The ecology of invertebrate fauna of Manx streams in relation to pollution from disused mines

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THE ECOLOGY OF INVERTEBRATE FAUNA OF MANX STREAMS IN RELATION TO POLLUTION FROM DISUSED MINES

Thesis submitted to the
Department of Biology of the Open University
in accordance with the regulations for the
degree of Doctor of Philosophy

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THE FLOW OF THE RIVER IS CEASELESS
AND ITS WATERS NEVER THE SAME.

Kamo No Chomei 1153 - 1216

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ABSTRACT

This investigation was carried out to inquire into the differences in the invertebrate fauna of two closely related streams in the Isle of Man.

The Gleneedle stream is a clean mountain stream which drains the western slopes of Gleneedle.

The Glendhoo stream drains the eastern sides of Gleneedle and collects runoff water from disused mine workings, it is slightly polluted by the presence of lead (mean annual concentration 0.26 mgl^{-1}) and zinc (mean annual concentration 0.108 mgl^{-1}).

The two streams combine and the sampling areas are about 400 metres apart at an altitude of approximately 150m.

The major components of the invertebrate fauna were determined for each stream, and observations of population changes recorded for a period of two years, from February, 1978, to January, 1980. Variations in the ionic concentration of lead and zinc, together with other chemical and physical parameters were also recorded.

Laboratory experiments were carried out to determine the hatching success of eggs of the mayfly *Baetis rhodani* and of the trichopterans *Hydropsyche instabilis*, *Rhyacophila dorsalis*, *Polycentropus flavomaculatus* and *Potamophylax latipennis*, incubated in water from the Gleneedle stream as a control, in water from the Glendhoo stream and in water to which had been added salts of lead and zinc.

Observations were also made in the laboratory of the effect of rearing larvae of *Potamophylax latipennis*, *Polycentropus flavomaculatus*, nymphs of *Baetis rhodani* and the plecopteran *Protonemura meyeri* in conditions similar to those of the hatching experiments.

Attempts to reproduce these experiments in the field did not succeed.

The results suggest that small amounts of lead and zinc of the order of 0.15 mgl^{-1} act synergistically to prevent the hatching of aquatic insect eggs; whilst these amounts are not fatal during larval instar stages they appear to be lethal during metamorphosis. Protonemura meyeri and Baetis rhodani were unable to moult successfully and failed to survive. The trichopteran larvae Polycentropus flavomaculatus and Potamophylax latipennis were resistant to lead and zinc and survived successfully in this stage. The rate of pupal emergence was low for Potamophylax latipennis and those teneral adults which did emerge had underdeveloped wings and were incapable of flight.

The existence of lead and zinc in the Glendhoo waters exerts a controlling influence on the ecology of the stream, colonization by insect larvae is prevented due to the eggs failing to hatch. The presence of P. latipennis larvae in the Glendhoo stream may be explained by the ovipositing behaviour of the imagines, eggs hatch on vegetation over-hanging the water. The larvae of this species are therefore able to inhabit the stream but development is inhibited during pupation.

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CHAPTER ONE

GENERAL INTRODUCTION

Physical and Historical Background

The Isle of Man is about 35 miles long by 12 miles wide (fig. 1.1.) it lies in the Irish Sea approximately 18 miles from Scotland, 28 miles from Ireland and 26 miles from England, and is often clearly visible from these adjacent coasts.

The Island has been described as "the Cumbrian Mountains in miniature ," it has a similar geological history to that area (Taylor, Land and Smith, 1971), which starts in the Ordovician period when the mud and siltstones of the Manx slate series were laid down within the confines of the subsiding Caledonian geosyncline. Deposition of mudstones and sandstones continued during the Silurian period and was interrupted at the beginning of the Devonian period by the onset of the Caledonian Orogeny, when periods of stability and deposition alternated with periods of intense folding. Igneous activity occurred during this period when the greatly crushed and contorted Manx slates were penetrated by intrusive material and became heavily mineralized.

The Caledonian Orogeny and its accompanying erosion created a new surface, basement conglomerate, on which sedimentation recommenced during Carboniferous times, these deposits are exposed at Castletown in the south of the Island as fossiliferous limestone containing a prolific coral and brachiopod fauna; and at Peel in the west of the Island, where an outlier of sandstone overlies the basement conglomerate.

During the Pleistocene period, the presence of the West British ice sheet considerably influenced the surface features of the Island. Erosion by melt water created many of the drainage channels in the southern uplands (Taylor, et al ,1971) but had little effect on the drainage pattern of the northern hills (Lamplugh, 1903).

With the withdrawal of the ice, raised beaches were exposed along the north west coast of the Island, and fluvio glacial drift material was deposited on the preglacial sea floor to form an addition to the Island, the northern plain.

During post glacial times, a thin layer of peat some two to three feet thick was laid down on the high ground of the N.W./S.E. central massif and winnowing of stratified glacial deposits resulted in the deposition of blown sand on the northern plain (Lamplugh, 1903).

Major depositions of metalliferous strata occur in the central southern uplands known as Foxdale, and in the Laxey area (fig. 1.2.). The principal ores are silver, lead, copper and zinc. Mining reached a peak during the late nineteenth century and during the period 1854-55 the output of zinc blende from the Laxey mine was greater than the combined output of all other mines in the British Isles (Mackay and Schnellman, 1963). Shortly after this, the output of the Foxdale group of mines declined, and all but one of the group ceased production. The remaining mine, the Foxdale mine, continued to be worked until 1918, when it ceased production (Kinvig,1971). Spoil heaps, known locally as "the deads" remain and exert a considerable impact on the surrounding area.

Cross's mine, fig. 1.3. (O.S. ref: SC 263781) and Dixon's mine fig. 1.3. (O.S. ref: SC 267782) (Garrad, Bawden, Qualtrough and Scratchard, 1972) are situated on the upper

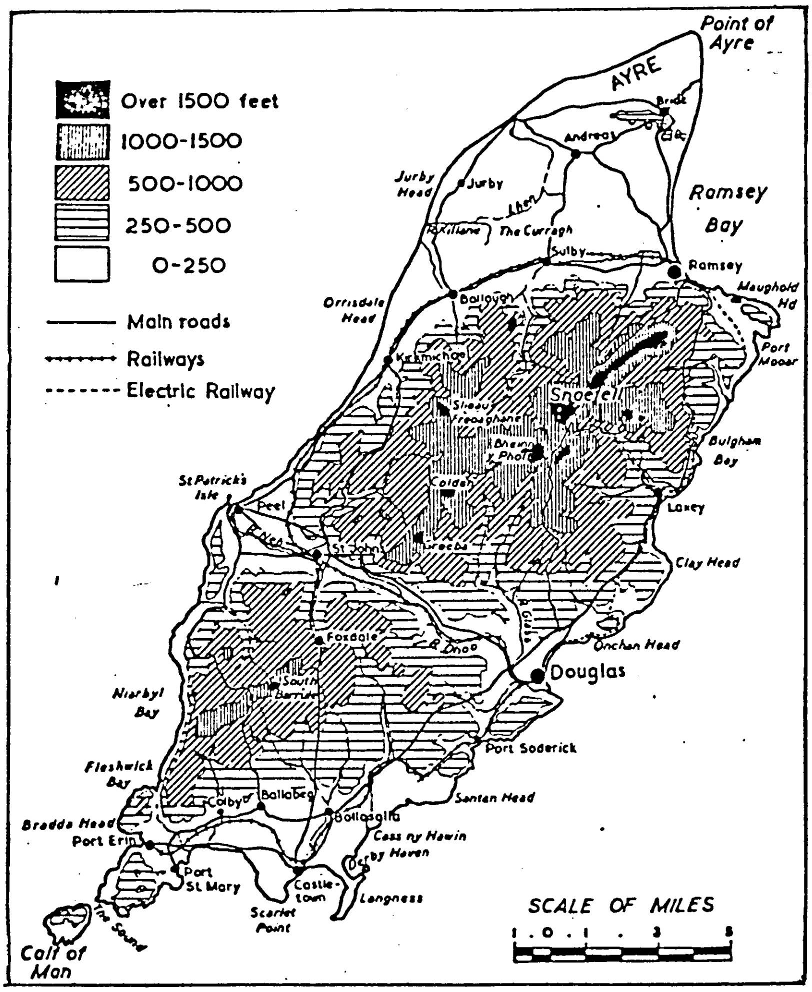


FIG. 1.1. The Isle of Man showing physical features (After Kinvig, 1971)

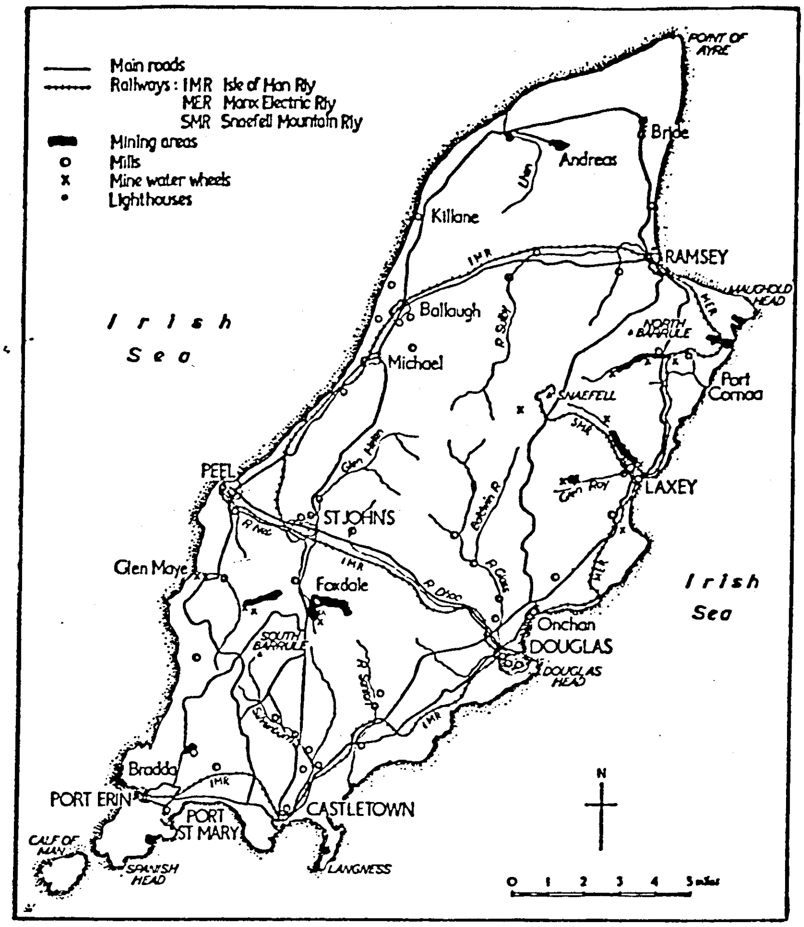


FIG. 1.2. The Isle of Man showing major mining areas.

(After Garrad, Bawden, Qualtrough and Scratchard, 1972)

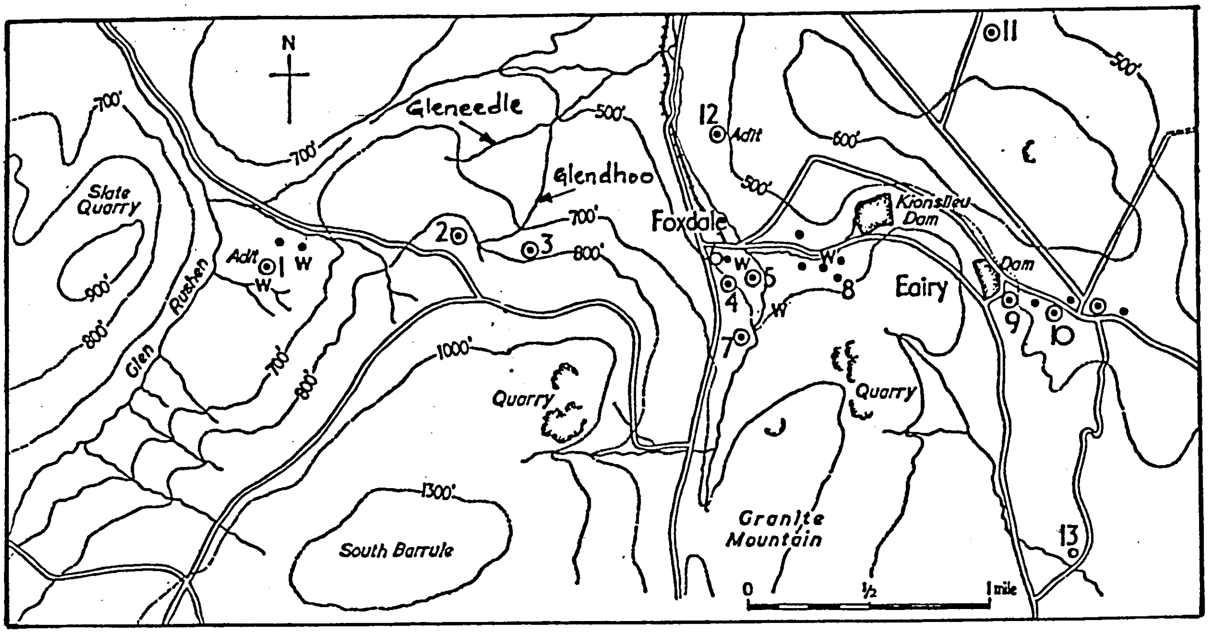


FIG. 1.3. Mines of the Foxdale area:

(1) Beckwith's; (2) Cross's; (3) Dixon's;

(4) Upper Old Foxdale; (5) Lower Old Foxdale;

(6) Old Flappy; (7) Maghie's; (8) Louisa;

(9) Far Gin; (10) New Foxdale; (11) Townshend's;

(12) Ballergy; (13) Ballanicholas;

(W) Water Wheel Site

(After Garrad, Bawden, Qualtrough and Scratchard 1972) eastern slopes of a small valley 2½ miles long and 1½ miles wide, known locally as Gleneedle. The western slope of the valley is free from mine workings, grazed extensively by sheep and cattle, and is drained by the Gleneedle stream.

Cross's mine has its spoil heap situated on a watershed, whilst the spoil from Dixon's mine was dumped on the banks of the Glendhoo stream. Adits from Dixon's mine also drain into the Glendhoo stream.

The Glendhoo and Gleneedle streams flow independently for about 1½ miles, then combine to form the River Foss, fig. 1.4. The areas chosen for investigation lie in each tributary about half a mile upstream from the confluence.

The Freshwater Fauna of the Isle of Man

There are many permanent small stony streams in the Isle of Man with rapid flow, and similar in character to those of the English Lake District (Hynes, 1941), and North Wales (Hynes, 1961). It would not be unreasonable to expect the freshwater fauna of these areas to be similar but this is not the case (Hynes, 1941; Pugh-Thomas, 1974).

Hynes (1952) during his investigation of the plecopteran fauna of the Isle of Man, sampled 66 lotic habitats, widely scattered over the Island, and records the presence of only 10 species of stonefly, listed in table 1.1. Hynes (1952) comments that the absence of _Perla carlukiana_ (Klapálek), _P. cephalotes_ (Curtis) and _Perlodes mortoni_ (Klapálek) is particularly striking as localities very suitable for these species occur frequently in the Island's rivers. The absence of _Isoperla grammatica_ (Poda), _Brachyptera risi_ (Morton), _Protonemura praecox_ (Morton) and _Capnia_ spp is also significant as these species are common in England, Ireland,

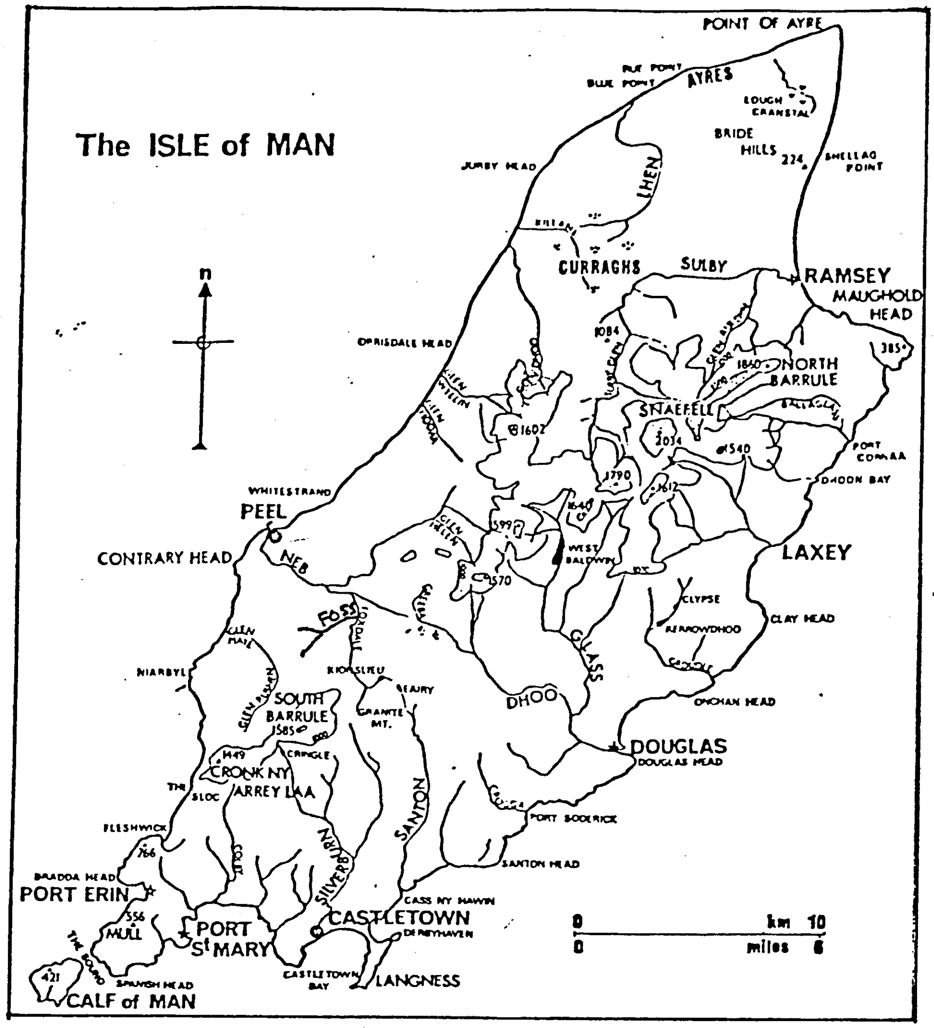


FIG. 1.4. The Isle of Man, showing major rivers and streams

(After Garrad, 1972)

FIG. 1.5. General View of Gleneedle

FIG. 1.6. View of the remains of mineworkings in the Gleneedle area showing Dixon's mine.

Diura bicaudata
Chloroperla tripunctata
Chloroperla torrentium
Leuctra hippopus
Leuctra inermis
Leuctra fusca
Protonemura meyeri
Amphinemura sulcicollis
Nemoura cinerea
Nemurella picteti

TABLE 1.1. The plecopteran fauna of the Isle of Man as recorded by Hynes (1952)

Paraleptophlebia submarginata
Ephemerella ignita
Caenis rivulorum
Baetis bioculatus
Baetis rhodani
Siphlonurus lacustris
Rhithrogena semicolorata
Heptagenia lateralis
Ecdyonurus venosus

TABLE 1.2. The ephemeropteran fauna of the Isle of Man as recorded by Cowin (1952)

Scotland and Wales (Hynes, 1952).

Cowin (1952) states that only 9 species of mayfly (Ephemeroptera) listed in table 1.2. have been recorded from Manx fresh waters, out of a total of 46 on the British list (Kimmins,(1972) gives a check list of 47 species and suggests that Caenis moesta was omitted from earlier lists). Pugh-Thomas (1974) however found only 4 species of mayfly in the Manx streams, recording Baetis rhodani as common in most running waters, Siphlonurus lacustris as rare and present in only one stream, Ecdyonurus venosus found in low numbers and Ephemerella ignita limited to locations with an altitude of less than 800ft.

Pugh-Thomas (1974) did not include trichopterans in his survey and says in his report "little work has been done on Trichoptera and this group would probably be an interesting study for future work." A search of the literature substantiates Pugh-Thomas's comments. However, Hynes (1954) when studying Gammarus in the Isle of Man included Trichoptera in his list of the faunal components of certain streams in the south of the Island.

Table 1.3 compares the species recorded by Hynes(1952, 1954), Cowin (1952), and Pugh-Thomas (1974), in Manx waters with an altitude of about 240m with those recorded by Hynes (1961) when investigating the Afon Hirnant, a soft water Welsh mountain stream of similar physical characteristics and altitude to the Manx streams.

Table 1.4. contrasts the faunal groups recorded in Manx streams of altitude approximately 240m with those recorded in certain mainland streams of similar altitude and characteristics; the Afon Hirnant (Hynes, 1961); the Shropshire Hill stream of Ashes Hollow (Arnold and Macan, 1969) and Llanycaiarn Brook, a tributary of the River Ystwyth

TAXONOMIC GROUP	GENUS	ISLE OF MAN	AFON HIRNANT
Coleoptera	Esolus	-	X
	Helmis	-	X
	Platambus	X	-
Crustacea	Asellus	-	X
	Gammarus	X	-
Diptera	Chironomids	X	X
	Simulium	X	X
Ephemeroptera	Baetis	X	X
	Ecdyonurus	X	X
	Ephemerella	X	X
	Heptagenia	-	X
	Rhithrogena	-	X
Hirudinea	Glossiphonia	-	X
	Erpobdella	-	X
Mollusca	Ancylastrum	X	X
Oligochaeta	Lumbriculus	X	X
Plecoptera	Amphinemura	-	X
	Chloroperla	X	X
	Diura	X	X
	Leuctra	X	X
	Nemoura	X	X
	Perlodes	—	X
	Protonemura	X	X
Trichoptera	Glossosoma	-	X
	Hydropsyche	X	X
	Philopotamus	-	X
	Plectrocnemia	-	X
	Polycentropus	X	X
	Rhyacophila	X	X
	Stenophylax = Potamophylax	X	X
TOTAL NUMBER OF GENERA		18	28

'X'indicates presence

TABLE 1.3. Comparison of the fauna of the Afon Hirnant and Manx streams of similar altitude and surroundings. Some genera given in tables 1.1. and 1.2. do not appear here because they occur only at low altitudes.
(After Cowin, 1952; Hynes, 1952, 1954, 1961; and Pugh-Thomas 1974).

TAXONOMIC GROUP	MANX STREAMS	AFON HIRNANT	ASHES HOLLOW STREAM	LLANYCHAIARN BROOK
COLEOPTERA	1	2	1	3
CRUSTACEA	1	2	2	1
DIPTERA	2	2	4	6
EPHEMEROPTERA	3	5	9	1
MOLLUSCA	1	5	3	3
OLIGOCHAETA	1	1	1	2
PLECOPTERA	5	7	9	3
TRICHOPTERA	4	7	7	7
TOTAL NUMBER OF TAXA	18	31	36	26

TABLE 1.4. Comparison of the major components of the fauna of the Afon Hirnant (a mountain stream in North Wales), Ashes Hollow stream, (a Shropshire hill stream) and Llanychaiarn Brook (mid Wales), with Manx streams of similar altitude. Some genera given in tables 1.1. and 1.2. do not appear here because they occur at low altitudes.
(After Arnold and Macan, 1969; Carpenter, 1924; Cowin, 1952; Hynes, 1952, 1954, 1961; and Pugh-Thomas, 1974).

in Mid Wales (Carpenter, 1924).

Examination of tables 1.3 and 1.4 indicates that the fauna of Manx streams is considerably impoverished when compared to similar streams on the adjacent mainland. It is interesting to note, in this context the unique distribution of the amphipods Gammarus duebeni and Gammarus pulex in Manx lowland streams. The Isle of Man is the only location in Britain where the two species of Gammarus occur together in fresh water, (Hynes, 1954).

It is a reasonable conclusion that the general poverty in the fauna of Manx streams may be ascribed to the geographical isolation of the Isle of Man (Hynes 1952, Pugh-Thomas 1974). Bailey (1908) states "the Manx fauna is derived from migrations across former land connections, the last bridge to exist being to the coast of Lancashire and Cumberland". Taylor, Land and Smith (1971) support this view and suggest that the severance of the land bridge to the English mainland during the final stages of the Pleistocene period has isolated the Island and contributed to the paucity of the post glacial Manx fauna, a view also supported by Lamplugh (1903).

There is always a large element of chance in the successful colonization of islands, organisms that may direct their passage, such as insects and birds, are frequently affected by adverse climatic factors such as unfavourable winds (MacArthur, 1972). Udvardy (1969) states that the role of a body of water in faunal dispersal is directly related to its size and permanence, thus making the sea a formidable biogeographic barrier. Thermal properties also add to the effectiveness of the sea as a barrier, the water being relatively cold even in the warmest seasons. Udvardy (1969)

In addition to the poverty of the Manx freshwater fauna, there is a paucity of literature of this subject. Pugh-Thomas (1974) writes in his report on freshwaters of the Island "the freshwaters of the Isle of Man are little known biologically in a British and also a European context and are of considerable scientific interest". This sentiment is echoed by other workers in the broader field of European freshwater invertebrate ecology (Hynes, 1977; Macan, 1973; Hickin, 1967), who frequently suggest that further work in this area is needed.

When there are few species in a stream, they are often represented by large numbers of individuals, so it might be expected that some Manx streams would have a fauna rich in number of individuals, though poor in number of species. The density of individuals must, however, depend upon environmental factors, including food supply.

It would be a reasonable assumption that two geographically close streams in the Isle of Man, of similar altitude, stream order, and physical conditions would have very similar freshwater fauna. Casual observations suggested that this was not so for the Gleneedle and Glendhoo streams, and investigation in greater detail confirmed that the fauna of the two streams was considerably different.

The purpose of this investigation was to examine the invertebrate macrofauna of the Gleneedle stream and the Glendhoo stream and to attempt to account for the observed differences in the ecology of the two streams.

CHAPTER TWO

CLASSIFICATION OF STREAMS

Introduction

In order to make a meaningful comparison between streams it is a necessary requirement that the collecting stations should be similar in physical, hydrological and ecological parameters. To enable these parameters to be assessed prior to the selection of suitable sites, river classification and zonation is briefly considered.

Stream Order

Strahler (1957) defines a first order stream as one which does not possess any tributaries and Shreve (1966) orders each link by the number of first order tributaries discharging into it. Thus a third order stream is comprised of three first order tributaries. The application of this classification is illustrated in fig. 2.1. based on the River Foss, the Gleneedle stream and the Glendhoo stream.

Classification by Biotic and Morphometric Parameters

Carpenter (1928) working on Cardiganshire streams described a typical river as having several sources in high ground, characterised by swift current velocities, steep gradient and erosion; as the current velocity decreases the river deepens, widens and deposits silt. Carpenter (1928) classified two primary zones, the highland brook and the lower courses, each sub-divided into stretches characterised by typical fauna as shown in table 2.1.

illustrates how the thermal properties of the sea effectively block the dispersion of free-swimming snakes from the Labrador coast to the Anticosti Island, and it is interesting to note that the Isle of Man is also devoid of snakes (Garrad, 1972). The presence of the sea will also hinder colonisation of islands by animals not able to swim, those without buoyancy, or those unable to tolerate the salinity; and a strong swimming land animal is unlikely to feed, rest or thermo-regulate effectively during a prolonged period in sea water. Small insects can often effectively cross a still water barrier by floating or utilising the surface tension (Udvardy, 1969), but this is unlikely to be an effective means of crossing the open sea owing to wave action. Udvardy (1969) also suggests that faunistic studies of land animals show that islands have a smaller, less balanced fauna, than adjacent mainlands, a view also shared by Pielou (1979).

Insect colonisation of islands is often accomplished by direct flight (Hynes, 1952) or by aerial drift (Hardy and Milne, 1937) and Pielou (1979) refers to this as "jump dispersal", however Andrewartha and Birch (1954) conclude that aerial drift is not an important mechanism in insect dispersal. In the case of the Isle of Man, colonisation by these methods seems improbable, due to the distances involved and the direction of the prevailing south westerly winds in the North Irish Sea (Isle of Man Meteorological Office) during periods of adult insect flight, especially for larger insecta such as Trichoptera, Plecoptera and Ephemeroptera. Andrewartha (1961) also suggests that large, strongly flying insects are frequently dispersed at low levels of up to 50ft by surface winds.

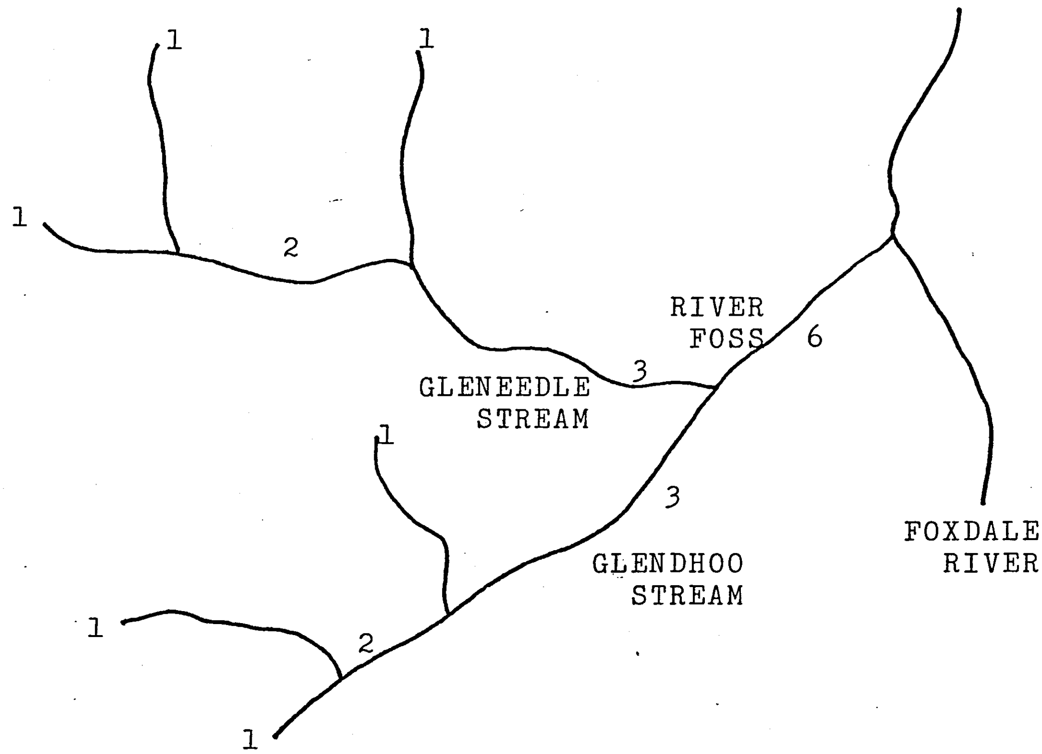


FIG. 2.1. Ordering of streams in the Gleneedle drainage area.

Arthropoda	
Crustacea	Canthocamptus, Asellus, Gammarus
Insecta	
Ephemeroptera	Baetis
Plecoptera	Leuctra, Nemoura, Isopteryx
Trichoptera	Adicella, Crunoecia, Agapetus, Stenophylax, Apatania, Tinodes
Coleoptera	Elmis, Anacaena
Arachnida	Hydracarina, Tardigrada
Platyhelminthes	
Turbellaria	Planaria alpina, Polycelis cornuta

TABLE 2.1. Invertebrate fauna of a typical head stream (After Carpenter, 1928)

Coelenterata	Hydra
Platyhelminthes	
Turbellaria	Planaria, Polycelis
Annelida	
Hirudinea	Glossiphonia, Erpobdella, Helobdella
Arthropoda	
Crustacea	Asellus, Gammarus
Insecta	
Ephemeroptera	Ecdyonurus
Plecoptera	Nemoura
Trichoptera	Hydropsyche, Rhyacophila, Glossosoma, Polycentropus, Plectrocnemia, Stenophylax, Agapetus, Brachycentrus,
Diptera	Simulium, Liponeura, Tanytarus, Orthocladius, Cricotopus
Mollusca	Ancylastrum, Lymnaea

TABLE 2.2. Invertebrate fauna of a typical trout beck (After Carpenter, 1928).

Carpenter (1928) described head waters as cold, oligotrophic, shallow streams having small volumes of water saturated with oxygen, the major insect fauna comprising Ephemeroptera (Baetis), Plecoptera (Leuctra, Nemoura, Isopteryx), Trichoptera (Agapetus, Stenophylax = Potamophylax) and Coleoptera (Elmis).

Trout becks were described by Carpenter as being formed by the union of head streams to give a stream having a more defined course and greater volume than head waters. The dominant ecological features being strong current, rocky bottom, and a sparseness of vegetation, these waters are also cold and saturated with oxygen. Characteristic fauna comprise Ephemeroptera (Ecdyonurus), Trichoptera (Hydropsyche, Rhyacophila, Stenophylax = Potamophylax, Polycentropus, Glossosoma, Plectrocnemia), Mollusca (Ancylastrum), and Diptera (Simulium) - table 2.2.

Huet (1954) classified river zones, by the key fish species present in each zone, but he extended the description by including the morphometric parameters characterising the different zones. Huet considered gradient to be the primary feature affecting such factors as temperature, current, velocity, and hence type of bottom, vegetation and benthic community fig. 2.2. Huet formulated a 'slope rule', fig.2.3. which demonstrates a relationship between river gradient, river width, type of bottom and fish fauna present. The slope rule suggests that stretches of rivers of similar physical characteristics such as depth, width and slope are likely to have similar fish populations. Thus it should be possible using Huet's system to predict the faunal zone of a river from a knowledge of its gradient and width.

BIOTIC ZONE	CHARACTERISTICS	CLASSIFICATION OF CURRENT VELOCITY
1	Stones more or less overgrown with moss or algae. Current velocity strong to moderate	Very strong $> 0.1\ m.s^{-1}$
2	Stony sandy brooks with accumulation of leaves at the bottom in places. As the bottom is variable, so is the current velocity from chiefly moderate, but sometimes strong, to very slight.	Strong: 0.05 to $0.1 m.s^{-1}$ Moderate: 0.25 to $0.5 m.s^{-1}$ Slight: 0.01 to $0.025\ m.s^{-1}$
3	Plants and submerged vegetation. Few stones Current velocity chiefly moderate to slight.	Very slight $< 0.01 m.s^{-1}$

TABLE 2.3. Classification of lotic biotopes.

(After Berg, 1948)

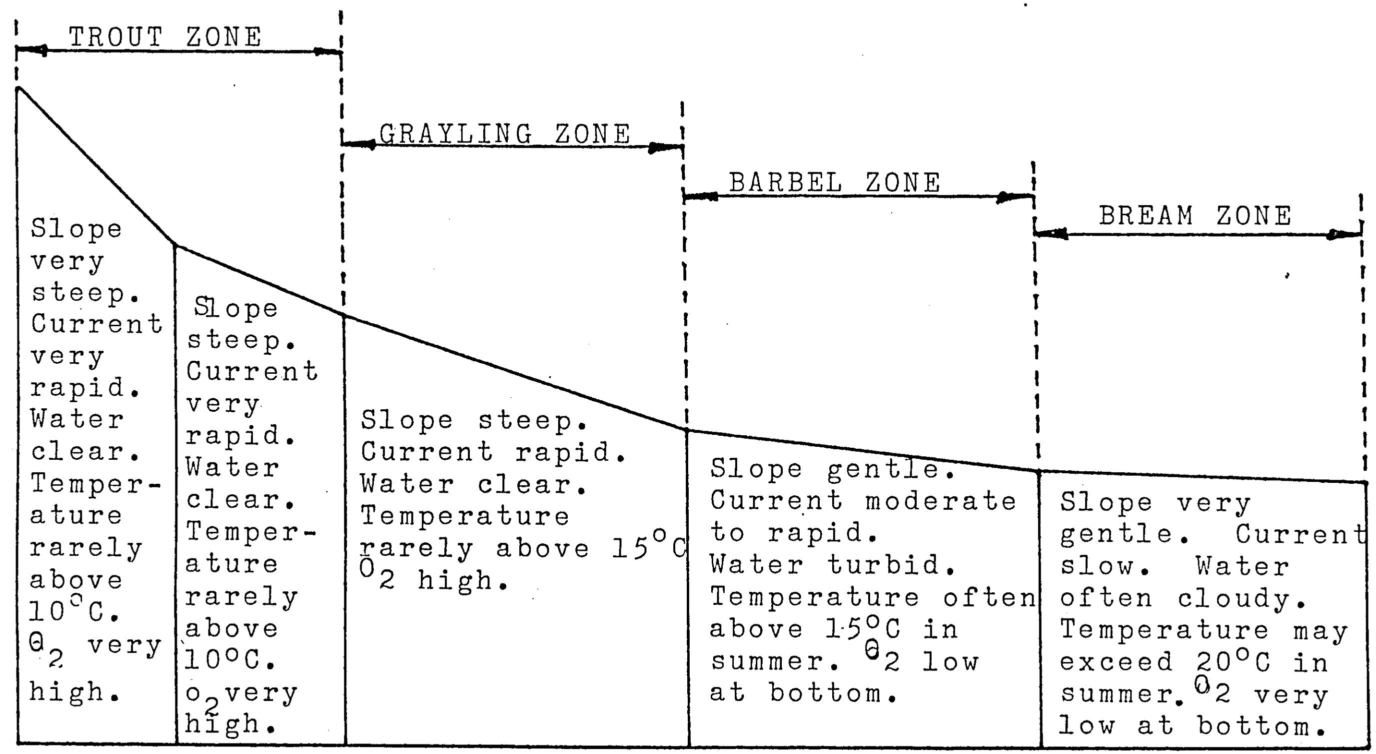


FIG. 2.2. Diagram showing relationship between stream gradient, temperature, current velocity and dissolved oxygen concentration (O_2), and Huet's classification of river zone.

(After Huet, 1954)

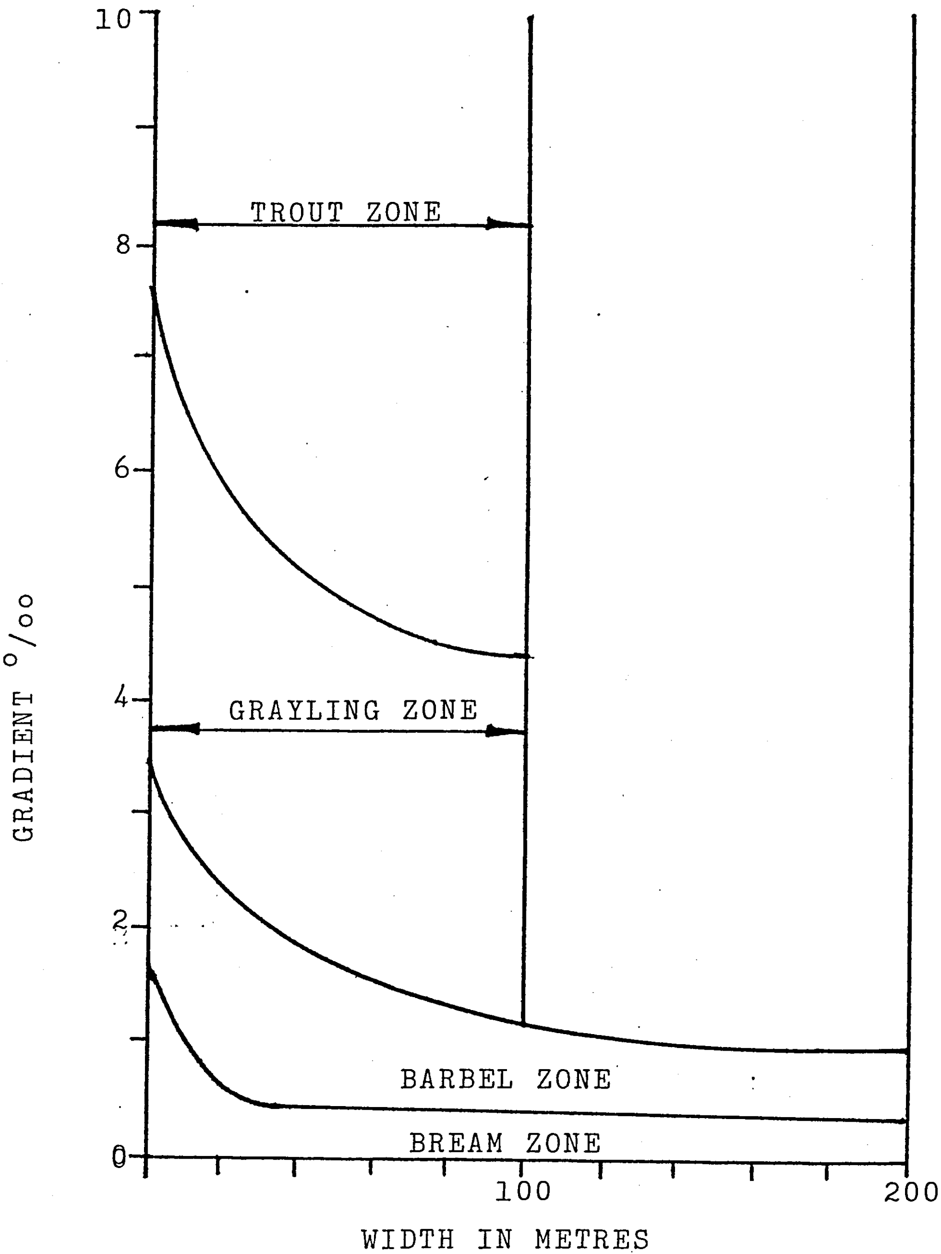


FIG. 2.3. Slope rule showing the relationship between gradient, width and fish zone. (After Huet, 1954)

ORDER	FAMILY	
	RHITHRON	POTAMON
EPHEMEROPTERA	Ecdyonuridae Ephemerellidae Leptophlebiidae	Potamanthidae Caenidae Polymitarcidae Siphlonuridae
PLECOPTERA	Capniidae Leuctridae Nemouridae Gripopterygidae	Perlidae Perlodidae
DIPTERA	Blepharoceridae Simuliidae Podonomidae Psychodidae	Culicidae Chironomidae Tabanidae Stratiomyidae
COLEOPTERA	Psephenidae Hydraenidae Elmidae Helodidae	Haliplidae Dytiscidae
TRICHOPTERA	Glossosomatidae Philopotamatidae Rhyacophilidae Odontoceridae	Hydroptilidae Leptoceridae
HYDRACHNELLAE	Protzidiae Hygrobatidae	
HETEROPTERA	-	Corixidae Notonectidae

TABLE 2.4. Fauna associated with rhithric and potamic river zones.

(After Illies, 1961)

ILLIES (1961)	HUET (1954)	BERG (1948)	CARPENTER (1928)
-	-	1	Head stream
Epirhithron Metarhithron	Trout zone	2	Trout beck
Hyporhithron	Grayling Zone	3	Minnow reach

TABLE 2.5. Comparison of some classification zones of upper reaches of a watercourse.

Berg, (1948) considered that the biotope was the basic ecological unit in the study of river ecology, and distinguished eleven such units, of which eight are appropriate to lotic waters and three to lentic environments. Only two of these units, biotic zones 1 and 2, listed in table 2.3. are relevant to this study.

Macan (1961) proposed a classification on the basis of indicator organisms (e.g. Ephemeroptera) while Lagler (1949) suggested that rivers having a faunal density greater than five hundred animals per square metre and a biomass greater than twenty one grammes, be classified as rich, and those with less than five hundred, and biomass smaller than eleven grammes, as poor.

Illies (1961), on the basis of his work on the River Fulda, Germany, distinguished between two primary zones, the upstream rhithron, and the downstream potamon. The rhithron was defined by Illies as that part of the river from the source, to a point where the mean temperature does not exceed 20°C, current velocity is high and volumetric flow small. Associated with the rhithron are cold, stenothermal, rheophilic organisms and highly oxygenated waters. These areas are characterised faunistically in table 2.4. Illies further divided the rhithron into epi, meta, and hyporhithron.

The classifications of Illies (1961), Huet (1954), Berg (1948) and Carpenter, (1928) are compared in table 2.5.

Classification of Manx Sites used as Collecting Stations

The Glendhoo and Gleneedle streams collect water from the eastern and western slopes respectively of the Gleneedle Valley. The valley runs approximately N.W./S.E. and is exposed to the predominant S.W. winds. The drainage area of

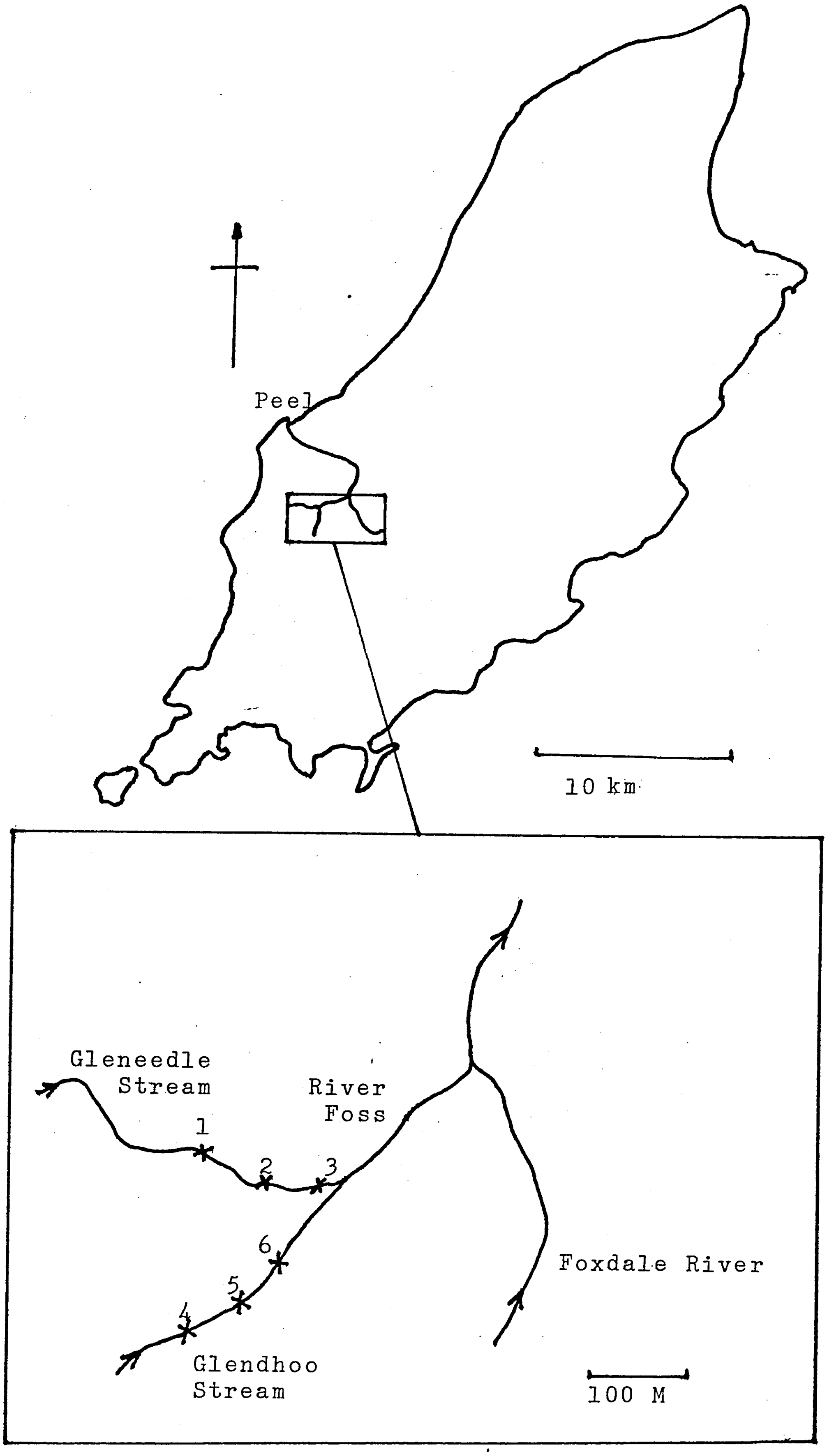


FIG. 2.4. Location of collecting sites.

the valley is approximately 3 km^2 and has an average mean annual rainfall of 115 cm, (data collected over a 20 year period, and supplied by the Isle of Man Meteorological Office) The Gleneedle stream rises at SC 258788 at an altitude of 200m and flows for 2.6 km before its confluence with the Glendhoo stream. The Glendhoo stream rises at an altitude of 225m at SC 266782 and flows for 2.8 km before joining the Gleneedle stream to form the sixth order River Foss as shown in fig. 2.1. (The stream names do not appear on the ordnance survey map of the Isle of Man, but are in current use by local residents). Both streams run for 500m from their source and cut deep channels through the peaty ground. There are no trees bordering the first 800m. The streams then descend steeply for the rest of their short length through a series of rocky channels and pools for about 1.5 km. At this stage in their development both streams have a mean width of 2m with riffles of average depth 15cm having beds of stones varying in size from 6 cm to 16cm diameter. The streams at this point are in the region of 1.9 km from their source and are bordered by extensive growths of *Salix fragilis*, grasses such as *Festuca*, and *Agrostis*, together with patches of *Calluna* and *Vaccinium*. Leaves of *Salix* can be found in the more sheltered points of the streams in various stages of decay, at all times of the year. At a distance of about 2.5 km from their respective sources, the streams have descended to an altitude of 105m and have each cut deep gouges out of the surrounding bedrock of the Manx slate series. There are frequent pools, varying in depth up to 5m, along the course of the streams, both waters being devoid of macrophytes and each having small colonies of the moss *Cinclidotus mucronatus* growing on stones at the edges of pools and riffles. Algae

STREAM	COLLECTING STATION		CURRENT VELOCITY M.S.$^{-1}$		TEMPERATURE °C		DISSOLVED OXYGEN % SATURATION		NATURE OF BED	PARTICLE SIZE CM RANGE	STREAMSIDE VEGETATION	EPILITHIC PERIPHYTON
	NO.	TYPE	RANGE	MEAN ANNUAL	RANGE	MEAN ANNUAL	RANGE	MEAN ANNUAL				
GLENEEDLE	1	RIFFLE	0.04 - 0.30	0.16	4-16	9.2	80-95	85.8	ANGULAR STONES	5-150	Salix fragilis	Rivularia spp. Cinclidotus spp.
	2	RIFFLE	0.04 - 0.28	0.13	4-16	9.2	80-96	86.1	ANGULAR STONES	5-155	Salix fragilis	Rivularia spp. Cinclodotus spp.
	3	RIFFLE	0.04 - 0.24	0.12	4-16	9.2	81-95	85.7	ANGULAR STONES	5-160	Salix fragilis	Rivularia spp. Cinclidotus spp.
GLENDHOO	4	RIFFLE	0.02- 0.26	0.13	4-16	9.2	80-96	86.1	ANGULAR STONES	5-160	Salix fragilis	Rivularia spp. Cinclidotus spp.
	5	RIFFLE	0.03 - 0.28	0.16	4-16	9.2	81-96	85.7	ANGULAR STONES	10-180	Salix fragilis	Rivularia spp. Cinclidotus spp.
	6	RIFFLE	0.02 - 0.21	0.11	4-16	9.2	80-94	85.8	ANGULAR STONES	7-175	Salix fragilis	Rivularia spp. Cinclidotus spp.

TABLE 2.6. Environmental and physical parameters of collecting stations on the Gleneedle and Glendhoo streams.

Note: Current velocity range includes dry weather flow and flood conditions.

were present on many stones above 7cm diameter, Rivularia being common among the epilithic periphyton.

The sides of the valley contain pasture land for cattle and sheep are grazed on the rougher Calluna covered areas.

The sampling sites, shown in fig. 2.4. were carefully selected to ensure that environmental factors such as light, aspect, streamside vegetation, current velocity, substratum and altitude were, as far as possible, similar at each collecting point.

Three sampling stations were used on each stream; each of these stations would be classified as rhithron by Illies' (1961) criteria, biotype No.2 using Berg's (1948) system and as trout becks by Carpenter's (1928) classification. Table 2.6. lists and compares these environmental parameters.

Both the Gleneedle stream and the Glendhoo stream are small, stony watercourses with clear waters and rapid flow. As the two streams are similar in physical appearance, nature of bed, gradient and flow velocity (Table 3.6.) similarities in the fauna, typical of small Manx streams (Pugh-Thomas 1974) could also be expected.

CHAPTER THREE

METHODS

Introduction

A repetitive sampling programme was carried out with samples collected at monthly intervals, commencing in February 1978 and ending in January, 1980.

Measurement of Chemical and Physical Parameters

(a) Field Measurements

pH, dissolved oxygen and water temperature were measured on site using a portable battery operated meter. Three readings were taken at each sampling station and the mean value recorded.

Current velocity was measured by timing a float passing over a measured distance of one metre at each sampling station. The mean value of five successive measurements at each sampling station was recorded, together with the maximum and minimum value. This method proved to be easy to carry out and provided a useful comparison. Scott (1958) also found that this simple method yielded useful results on the River Dean, but Cummins (1962) considers that the method does not provide useful information about the microdistribution of benthic animals, and records that few workers have measured velocity at the stream bottom where it influences animal distribution.

(b) Laboratory Measurements

Two water samples were taken on each occasion from the collecting stations using 500 ml plastic containers. Both containers were filled to the brim to avoid gaseous exchange. One sample was acidified with 2 ml of concentrated nitric acid to prevent adsorption of metal ions on the container walls. This sample was used to measure the concentration of lead and zinc ions in solution using a Pye Unicam S.P. 90A series 2 atomic absorption spectrophotometer, and standard blank solutions of known ionic concentration, from one to twenty milligrammes per litre, for comparison. The second sample was used in the laboratory to confirm pH and dissolved oxygen concentration, previously recorded in the field.

The standard chemical procedures used throughout this investigation are those reviewed by Golterman, Clymo and Ohnstad, (1978); Klein, (1959); and Mackereth, Herm and Talling, (1978) (see also appendix).

Water samples, sieved through a mesh of 300 microns aperture, and sediment samples were also taken at irregular intervals from each collecting site to the laboratory. These water samples were used to determine the five day biochemical oxygen demand, nitrate, calcium, magnesium and sodium concentrations using atomic absorption spectrophotometry, and standard chemical and collecting methods described by Golterman et al. (1978). The sediment samples after digestion in nitric acid, were used to assess the amount of zinc and lead present using atomic absorption spectrophotometry. Samples were collected during flood, dry weather and normal flow conditions. Details of the chemical analysis are described more fully, in the appendix.

Biological Sampling

Preliminary observations had shown the macrofauna of the Gleneedle and Glendhoo streams to be mainly the cased caddis *Potamophylax*, the net spinning *Hydropsyche* and the free living *Rhyacophila*, together with stone flies *Nemoura* spp and the may fly *Baetis*. All except *Baetis* tended to have a contagious distribution (Elliott,1977) in the Gleneedle stream, and only Potamophylax was common to both waters.

The literature on benthic sampling is listed by Elliott and Tullett(1978) in their bibliography, and various techniques are evaluated by Cummins (1962). Macan (1958) lists five categories of sampling methods suitable for investigating the bottom fauna of stony streams: hand lifting of individual stones, provision of substratum for colonization, boxes and cylinders, fixed nets and moveable substratum nets. Macan suggested that hand lifting is the only method that can be used on a bottom consisting of large stones and that sampling be confined to fixed periods of time. Cummins (1962) does not consider this method quantitative in any strict sense, one of his objections being that the method does not retain sediment for physical analysis. However Macan is of the opinion that, although hand lifting is not a quantitative technique, it does yield comparable figures. This method was successfully adopted by Arnold and Macan (1969).

Various sampling techniques were tried at the start of this programme, the kick technique described by Hynes (1961) was used to sample for Baetis spp. but the technique of pressing a metal frame of known area into the stream bottom, utilized by Scott (1958) in the River Dean, was found to be unsuitable due to the nature of the stream bed.

Owing to the nature of the stream bottom, and the composition of the macrofauna, Macan's stone lifting technique was found to be the most suitable collecting method for an epibenthic plecopteran and trichopteran fauna. This method was carried out for a five minute period at each collecting site. Baetis was sampled by using Hynes kick technique for ten seconds with a standard F.B.A. pond net of 28.5cm diameter and 20 meshes per inch.

Animals were identified in the field using a X5 hand magnifier but for the first six months two individuals of each species were brought to the laboratory to confirm the initial identification using keys by Hickin (1967), Macan (1974, 1979), Hynes (1977), Armitage, Furse and Wright (1979), and Edington and Hildrew (1981).

To avoid depletion of Plecoptera and Trichoptera, all larvae were returned to the stream after field identification.

Potamophylax latipennis is easy to detect due to its habit of congregating in numbers on the underside of large flat stones. Old cases, still attached to stones, were full of silt, but cases recently vacated by hatched teneral adults were recognisable because of the absence of silt, these were removed from the stones (but not included in the sample count). Identification was achieved by dissecting the stone case to reveal sclerotized parts of the larvae discarded during pupal metamorphosis. The diagnostic parts were the head capsule, in particular the clypeus, gular and anal sclerites, the terminology is that used by Hickin (1967). Mackereth (1954) refers to the clypeus as the fronto clypeus, but Edington (1964) uses the term fronto clypeal apatome, Mackereth's terminology will continue to be used in this study.

Before examination each trichopteran head capsule was cleaned by soaking in a 2% solution of potassium permanganate for 24 hours; this enabled the markings to be distinguished on the fronto clypeus and genae.

The data obtained are presented and discussed in the following chapters and are fully tabulated in the appendix.

CHAPTER FOUR

INVERTEBRATE POPULATIONS

Results

The components of the invertebrate epibenthic macrofauna were established, for both the Gleneedle and the Glendhoo streams, during preliminary surveys carried out in June, September and December, 1977. Timed sampling methods and random sampling programmes were not employed during these preliminary investigations because the streams are small enough to permit the whole of the lotic environment to be sampled at each station.

The composition of the major components of each stream are shown in table 4.1. Sixteen species were recorded in the Gleneedle stream but only four species were found in the waters of the Glendhoo stream.

Seasonal fluctuations in numbers, recorded in figs. 4.1. to 4.6. are clearly evident in the macroinvertebrate populations of both streams, with maximum densities occurring in the mid summer months. The populations for 1978 are consistently higher for each species in both waters than for 1979. This may be the result of the very heavy rainfall during November 1978, which caused the rivers to flood, (preventing the collection of invertebrate samples), and was the heaviest rainfall recorded for forty years (Isle of Man Meteorological Office).

The campodeiform trichopteran larvae <u>Polycentropus flavomaculatus</u>, <u>Hydropsyche instabilis</u>, <u>Rhyacophila dorsalis</u> together with nymphs of the plecopteran <u>Protonemura meyeri</u> and the ephemeropteran <u>Baetis rhodani</u> were only found in the waters of the Gleneedle stream, the most numerous being

TAXONOMIC GROUP	SPECIES	GLENEEDLE STREAM	GLENDHOO STREAM
Diptera	Simulium spp	XXX	X
Arachnida	Hydrachnellae	X	-
Hemiptera	Velia caprai	XX	XX
Coleoptera	Platambus maculatus	X	X
Trichoptera	Potamophylax latipennis	XXX	X
	Rhyacophila dorsalis	X	-
	Hydropsyche instabilis	X	-
	Polycentropus flavomaculatus	X	-
Ephemeroptera	Baetis rhodani	XXXXX	-
	Baetis tenax	X	-
	Ephemerella ignita	X	-
	Ecdyonurus dispar	X	-
Plecoptera	Protonemura meyeri	X	-
	Nemoura cambrica	X	-
	Diura bicaudata	X	-
Mollusca	Ancylastrum fluviatile	XXX	-
Total number of species present		16	4

'X' indicates presence and relative abundance

TABLE 4. 1. Composition of the invertebrate macrofauna of the Gleneedle and Glendhoo streams.

Baetis rhodani. Larvae of _Rhyacophila dorsalis_, _Polycentropus flavomaculatus_ and _Hydropsyche instabilis_ were always present in the Gleneedle stream. _H. instabilis_ appeared in maximum numbers in March and April, whilst _Polycentropus flavomaculatus_ populations were greatest in May with _Rhyacophila dorsalis_ being most numerous in June.

Velia caprai, the surface dwelling hemipteran was the only species present in similar numbers in both streams, and the eruciform trichopteran, _Potamophylax latipennis_, was the only member of its order that was present in both waters, but the numbers were fewer in the Glendhoo stream.

Seasonal fluctuations of _Potamophylax latipennis_ in the Gleneedle and Glendhoo streams are shown in fig. 4.5. and 4.6. respectively (and also in appendix table A1). The populations follow a broadly similar pattern of seasonal variation over the year, but the density of _P. latipennis_ was significantly less ($P<0.1$, see appendix) in the Glendhoo stream than the Gleneedle stream. Pupae were not included in the figures, but it was noted that pupal cases with partially developed dead pupae were found only in the Glendhoo stream. The number of these undeveloped pupae are marked on fig. 4.6. they were always found to be covered by a fine whitish grey bacterial growth, possibly _Sphaerotilus natans_ (Hynes, 1960) and when the case was removed from the attached stone, they had a fetid odour. Laboratory investigations indicated that the pupae were either fully or partly decomposed.

Discussion

When Hynes (1952) studied the plecopteran fauna of Manx streams, he recorded only one species _Protonemura meyeri_ from the head waters of Glen Rushen, a stream very similar in aspect, altitude and environment and only 2 km distant from the Gleneedle stream. The presence of Nemoura cambrica in the waters of the Gleneedle stream is also in contrast to the observations of Hynes (1952) who noted the absence of N. cambrica from Manx waters. The absence of Gammarus spp. from waters of these altitudes in the Isle of Man is also confirmed by earlier work by Hynes (1954).

The presence of Baetis rhodani in the Gleneedle stream at all times of the year is in contrast to that of the closely related B. tenax which was absent from the Manx stream during the winter. Pleskot (1958)also records the absence of B. vernus from Austrian streams during the winter months and Macan (1957a) noted the presence of B. rhodani throughout the year in Ford Wood Beck in the English Lake District. The life history of Baetis spp has been documented Macan (1957b), Elliott (1967), who suggests they are bivoltine, and Humpesch (1979). In contrast, Hynes (1961) presents evidence for B. tenax being univoltine. Macan (1979)also suggests that B. tenax is univoltine with an emergence period from late June to September, and distinguishes B. tenax from B. vernus only on habitat. Macan (1979)applied the names B. tenax to species taken at high altitudes and B. vernus to species taken at low altitudes. B. tenax has been recorded at eleven stations in highland Britain including the Lake District (similar in character and only 26 miles distant from the Isle of Man) ranging in altitudes from 350m to 900m (Macan 1979). The data presented in fig 4.4 was collected at an altitude of 150m, the species being classified as B. rhodani in accordance with Macan (1979).

The two streams are only 400m apart at the sampling sites and Potamophylax latipennis have been observed ovipositing in the Glendhoo stream so it is unlikely that the differences in population densities of this caddis can be attributed to the failure of P. latipennis to oviposit in this stream and incidentally it seems likely that other flying

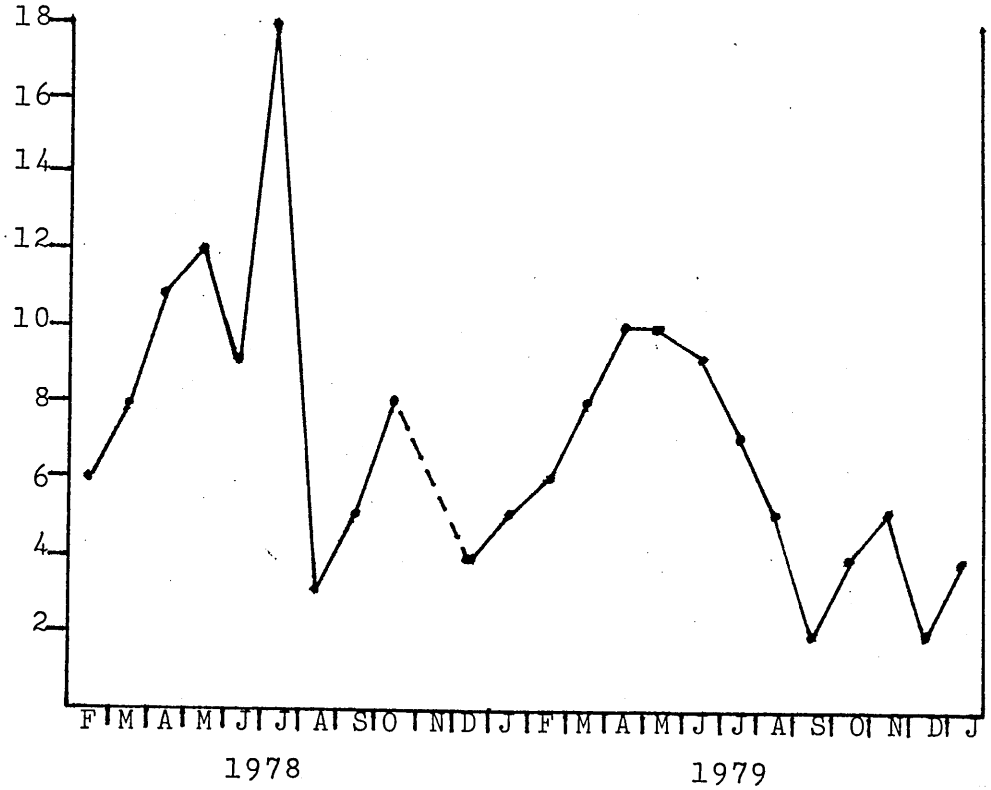


FIG. 4.1. Seasonal fluctuations in the total number of larvae of <u>Rhyacophila dorsalis</u> sampled from 3 collecting stations in the Gleneedle stream February, 1978 to January, 1980

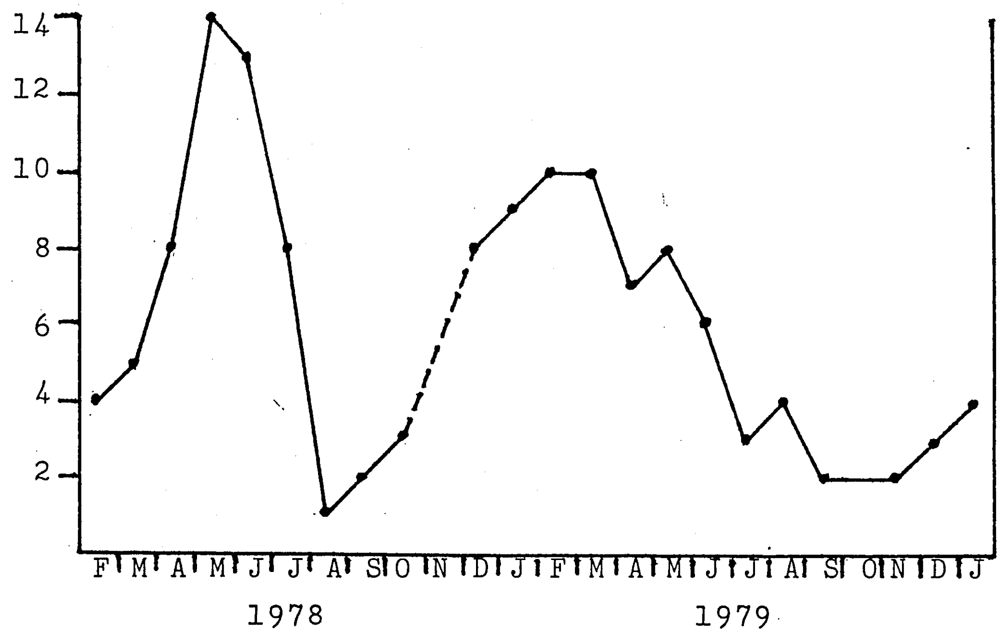


FIG. 4.2. Seasonal fluctuations in the total number of larvae of *Polycentropus flavomaculatus* sampled from 3 collecting stations in the Gleneedle stream February, 1978 to January, 1980

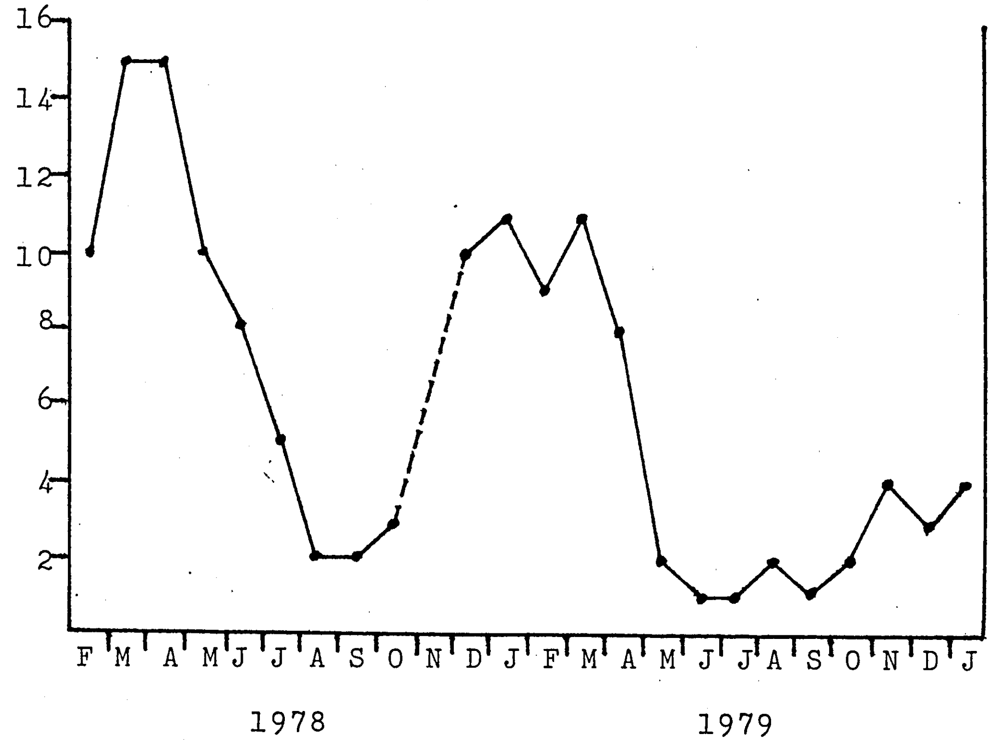


FIG. 4.3. Seasonal fluctuations in the total number of larvae of *Hydropsyche instabilis* sampled from 3 collecting stations in the Gleneedle stream February 1978 to January, 1980.

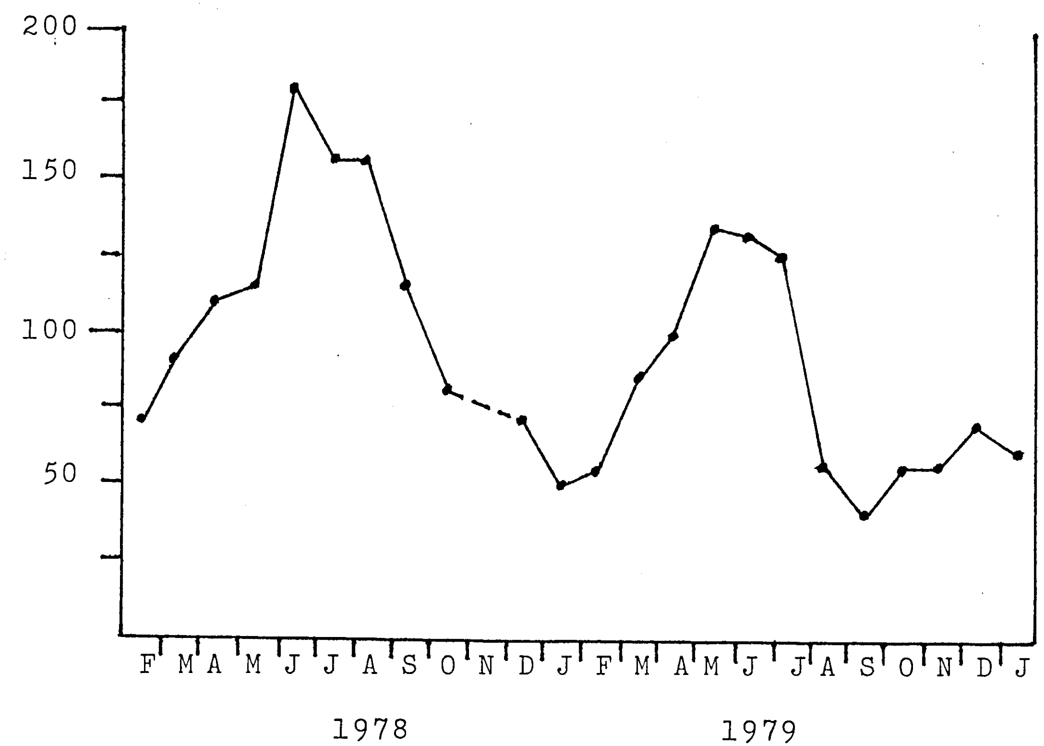


FIG. 4.4. Seasonal fluctuations in the total number of nymphs of *Baetis rhodani* sampled from 3 collecting stations in the Gleneedle stream February, 1978 to January, 1980.

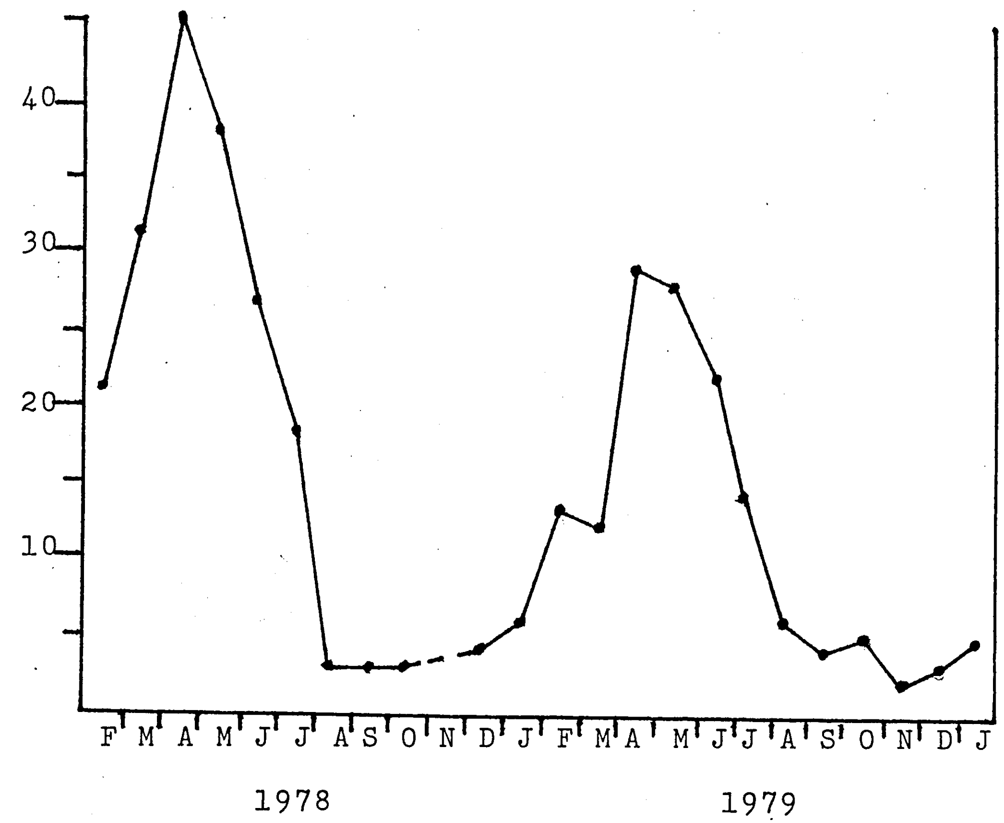
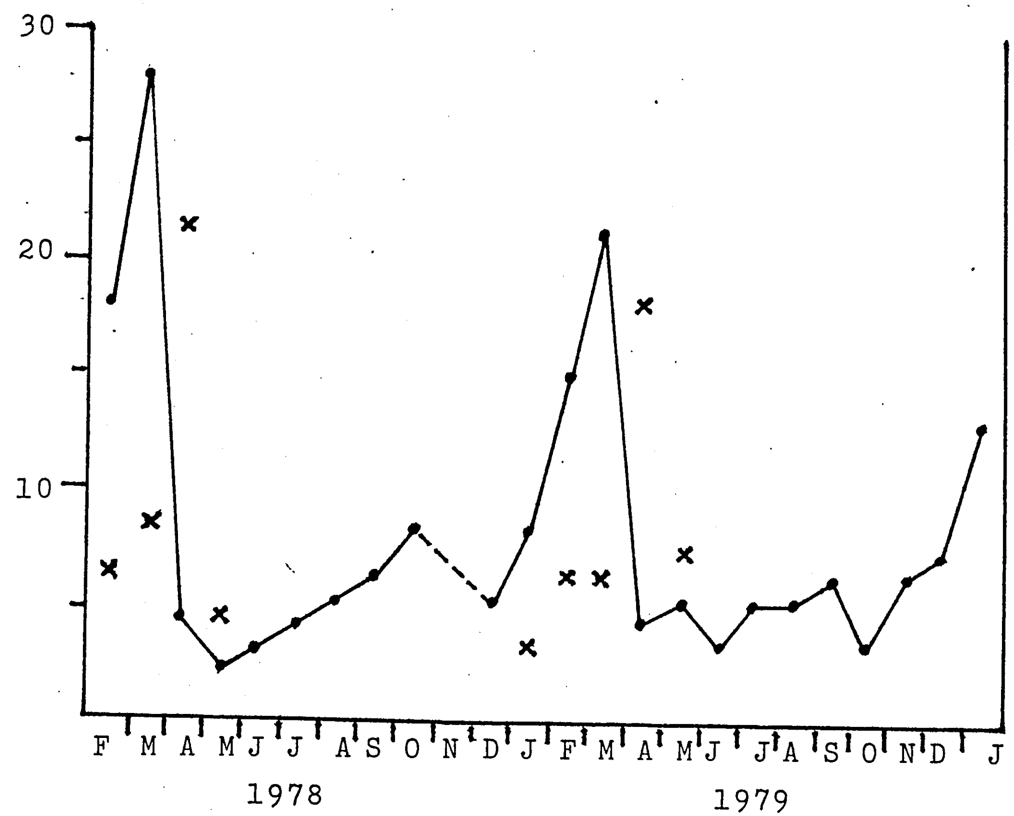


FIG. 4.5. Seasonal fluctuations in the total number of larvae of *Potamophylax latipennis* sampled from 3 collecting stations in the Gleneedle stream February, 1978 to January 1980.



NOTE: Lower limit of size for Figs. 4.1. to 4.6. was 3mm.

FIG. 4.6. Seasonal fluctuations in the total number of larvae of Potamophylax latipennis sampled from 3 collecting stations in the Glendhoo stream February 1978 to January 1980. 'X' indicates the number of cases found with dead pupae in them

insects that emerged from the Gleneedle waters could visit the Glendhoo stream. Comparison of figs. 4.5. and 4.6. suggest that for both years *P. latipennis* reached its maximum population density one month earlier in the Glendhoo stream, and there was then a sudden and dramatic decline in numbers, immediately following the maximum population density. This is in marked contrast to the Gleneedle stream where the population of *P. latipennis* declined much more slowly after the maximum.

The inability of *P. latipennis* to pupate successfully together with a complete absence of a plecopteran and ephemeropteran fauna in the Glendhoo stream, suggest that limiting factors could be lack of suitable food, competition for habitat or the presence of chemical factors in the waters.

Jones (1950) investigated the feeding habits of aquatic insects, taken from the River Rheidol, by examining their gut contents, he found that the ephemeropteran *Baetis* subsisted mainly on detritus and small amounts of algae, the plecopteran *Protonemura* fed mainly on detritus and dead leaves; the omnivorous trichopteran *Hydropsyche* consumed detritus, leaf fragments and *Simulium* larvae, whilst the carnivorous trichopterans *Polycentropus* and *Rhyacophila* fed upon a variety of insects such as *Velia*, *Simulium* larvae and members of their own species. Jones concluded that the macroflora, particularly diatoms, form an unreliable source of food; and suggested that detritus and dead leaves trapped between stones on the riffle are a much more reliable source of food for stream insects.

Food is unlikely to be a limiting factor in the Glendhoo stream because of the presence of *Simulium* larva, *Velia*, detritus and dead leaves. *Potamophylax latipennis* was observed to feed on *Salix fragilis* in laboratory conditions (see chapter six) and S. fragilis leaves were present in both the Gleneedle and Glendhoo streams at all times of the year. Competition for habitat is unlikely to be a controlling factor because Potamophylax latipennis is the only trichopteran present in the Glendhoo stream.

Roback (1974) lists the extreme tolerance of insect orders to some chemical factors: dissolved oxygen concentration, pH, biochemical oxygen demand and the presence of iron, see table 4.2. These tolerance limits are not exceeded in the Glendhoo stream (see Chapter Five) therefore, it is unlikely that the absence of the aquatic stages of insects can be attributed to them.

Carpenter (1924) reported the absence of Trichoptera and a much reduced plecopteran fauna in waters containing 0.2 to 0.5 mgl^{-1} lead; Jones (1958) and Brown (1977) reported an impoverished fauna, chiefly Ephemeroptera and Plecoptera in waters containing 0.2 to 0.7 mgl^{-1} zinc. This suggests that the presence of mines and spoil heaps could be important and their effects should be investigated.

However, Brooker and Morris (1980) investigated the macroinvertebrate riffle fauna of the rivers Rheidol and Ystwyth and reported that there was no evidence that the relative abundance of the fauna was related simply to heavy metal concentrations of up to 0.34 mgl^{-1} zinc in the Rheidol and up to 2.0 mgl^{-1} zinc in the Ystwyth. The insect fauna was dominated by Plecoptera and Ephemeroptera, Trichoptera contributed from 4 to 21% of the population density in the Rheidol but only 3 to 10% in the Ystwyth.

Nielsen (1974) states that Potamophylax latipennis was found in low numbers in the small but heavily polluted Danish River Lindenborg and that all pupae found were dead. Nielsen

ORDER	PH		Fe	DISSOLVED OXYGEN	B.O.D.	
	<4.5	>8.5	>5.0 mgl^{-1}	<4 mgl^{-1}	>5.9 mgl^{-1}	>10.0 mgl^{-1}
Ephemeroptera	0	3	0	2	14	1
Trichoptera	1	8	1	2	9	0
Plecoptera	0	6	0	0	2	0
Diptera	5	13	3	15	8	5
Hemiptera	3	3	2	8	8	1
Coleoptera	9	14	5	10	9	0

TABLE 4.2. Summary of extreme tolerance by insect order. Figures show numbers of taxa in each order tolerating the limit.

Data adapted from Roback, (1974)

goes on to suggest that the presence of larvae in the river must be due to immigration of adults from nearby unpolluted streams. Nielsen's findings imply that P. latipennis is resistant to pollution in the larval stage but is susceptible during pupation, a situation which could have a counterpart in the Manx streams of Glendhoo and Gleneedle.

CHAPTER FIVE

PHYSICO-CHEMICAL FACTORS

Results

Water temperature showed seasonal variations from a winter minimum of 3.5°C to a maximum of 16°C during the summer months, to give an annual range in the region of 12°C and a mean annual value of 9°C. Temperatures were measured at three sites on each stream as near as possible to 2.00p.m. There were no significant differences in the temperature at each of the six stations on any one occasion (see appendix table A5). The temperatures obtained for the Gleneedle and Glendhoo streams (fig 5.1) are similar to those obtained by Hynes (1954) who recorded a minimum of 4.5°C and a maximum of 15°C for two streams in the south of the Island.

Measurements of dissolved oxygen, as a percentage of saturation, were also recorded (fig 5.2.) whilst the water temperature was being measured. There were no significant differences in the oxygen concentration of the six stations on any single sampling occasion (see appendix table A7). Dissolved oxygen was at a minimum during the summer months and reached near saturation maxima in the colder winter months.

The values of pH monitored on a monthly basis and recorded in fig 5.1 (see also appendix table A4) ranged from 5.9 to 6.3. These values were remarkably consistent throughout the period with no significant variation in the value of pH at each station on any one sampling occasion.

Biochemical oxygen demand, calcium, magnesium, sodium and nitrogen (as nitrate) concentrations were measured on six occasions, and listed in table 5.1. in order to compare the two streams. The values of these parameters were essentially the same in the Gleneedle and the Glendhoo streams ($P < 0.05$)

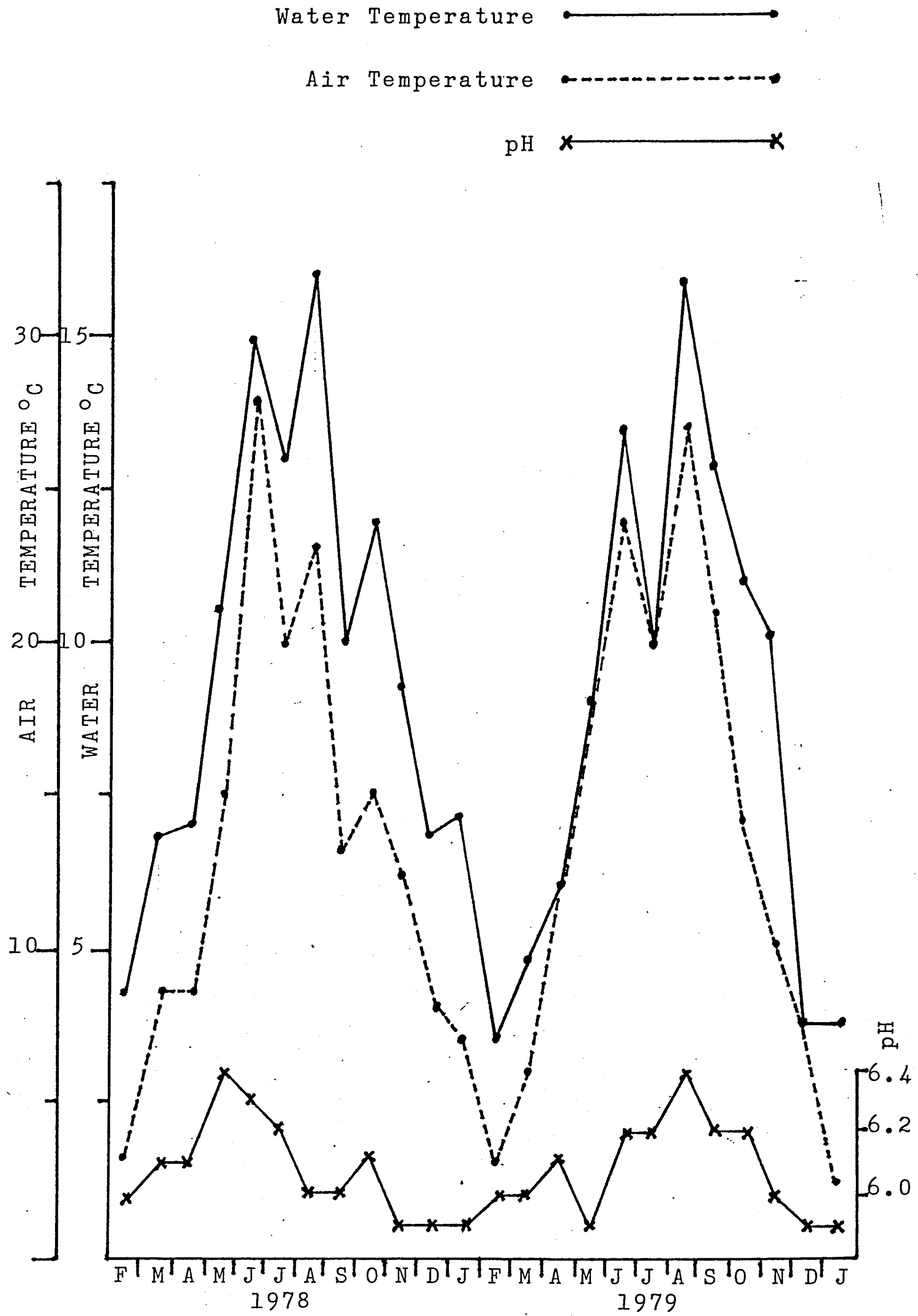


FIG. 5.1. Seasonal variations in pH, water temperature and air temperature for the Glendhoo and Gleneedle streams for February, 1978 to January, 1980.

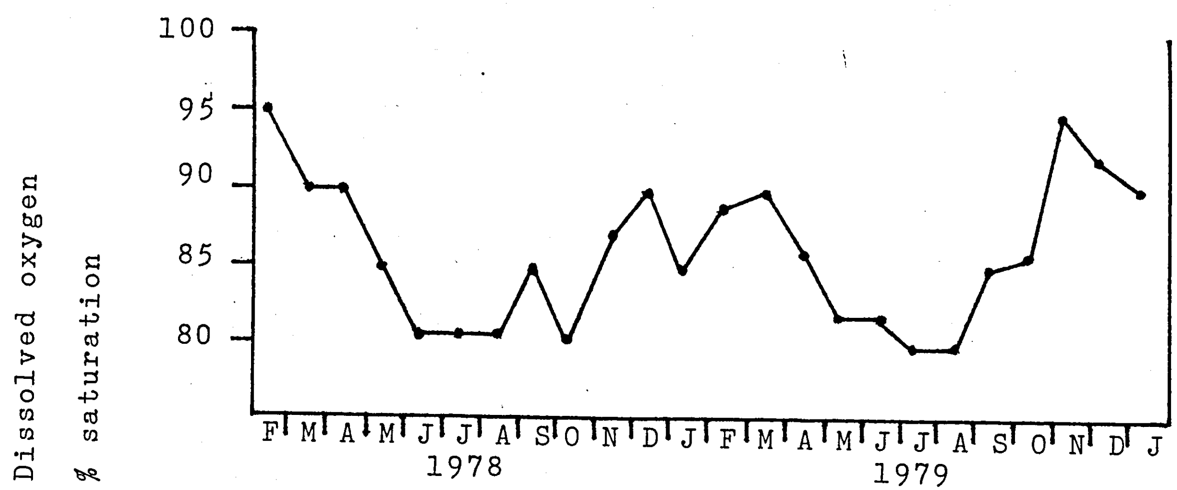


FIG. 5.2. Seasonal variations in dissolved oxygen content of Glendhoo and Gleneedle streams for February, 1979 to January, 1980.

STREAM	PARAMETER mg.l^{-1}	FEB 1978	JUL 1978	SEP 1978	JAN 1979	JUL 1979	OCT 1979
GLENEEDLE	B.O.D.	1.2	2.2	1.0	1.3	2.0	0.9
	NITRATE NITROGEN	0.003	0.004	0.016	0.002	0.004	0.020
	CALCIUM	3.0	6.0	2.5	3.5	6.5	2.0
	MAGNESIUM	3.0	6.0	2.5	3.5	6.0	2.0
	SODIUM	10.0	12.0	9.0	11.0	14.0	9.0
GLENDHOO	B.O.D.	1.3	2.1	0.9	1.3	2.1	1.0
	NITRATE NITROGEN	0.003	0.004	0.015	0.002	0.004	0.012
	CALCIUM	2.5	6.5	2.5	3.5	6.5	2.0
	MAGNESIUM	2.0	6.0	2.0	3.0	7.0	2.0
	SODIUM	10.0	12.0	9.0	11.0	14.0	9.0

TABLE 5.1. Variations in the biochemical oxygen demand (B.O.D.) nitrate nitrogen concentration and the concentration of calcium, magnesium and sodium at different times in the Gleneedle Glendhoo streams.

see appendix).

Water samples were taken from the Glendhoo stream at monthly intervals over a two year period from February, 1978 to January, 1980, and from the Gleneedle stream at monthly intervals for a one year period from February, 1978 to January, 1979.

Analysis of water from each stream indicated the presence of iron in both the Gleneedle and the Glendhoo streams, within the range 0.05 to 0.20 mgl^{-1} as listed in table 5.2. These amounts were not considered critical in view of the work by Doudoroff and Katz (1953) and Wood (1974) and iron concentrations were not monitored on a monthly basis. Copper was not detected in the waters of either stream but was present in small quantities in the sediments. The amounts of copper listed in table 5.3. are not considered significant.

Seasonal variations in the concentrations of lead and zinc, together with surface current velocity of the Glendhoo stream are shown in fig. 5.3. There is a positive correlation (τ = 0.66 see appendix) between the concentration of lead and zinc in solution and surface current velocity. The mean annual value for lead concentration was 0.26 mgl^{-1} with a range of 0.10 to 0.70 mgl^{-1}. The monthly variation was considerable, being approximately 69% of the mean value for the period of study. The range for zinc was 0.03 to 0.20 mgl^{-1} with a mean annual value of 0.108 mgl^{-1}, the variation being, on average, 62% of the mean value for the period.

Measurements of the concentrations of lead and zinc in the waters of the Gleneedle stream were discontinued after twelve months because the analysis from each site showed that these metals were not detectable in the water, within the limits

STREAM	CONCENTRATION OF IRON mgl^{-1}					
	FEB 1978	JUL 1978	SEP 1978	JAN 1979	JUL 1979	OCT 1979
GLENEEDLE	0.05	0.10	0.05	0.05	0.20	0.05
GLENDHOO	0.05	0.15	0.05	0.10	0.20	0.05

TABLE 5.2. Variations in the concentration of iron at different times in the waters of the Gleneedle and Glendhoo streams during a period from February, 1978 to October, 1979.

STREAM	METAL	CONCENTRATION mgl^{-1}					
		FEB 1978	JUL 1978	SEP 1978	JAN 1979	JUL 1979	OCT 1979
GLENEEDLE	LEAD	6	2	53	3	5	47
	ZINC	2	4	80	7	8	74
	COPPER	2	2	19	0	0	21
GLENDHOO	LEAD	800	670	1,200	810	680	950
	ZINC	2,000	1,000	2,500	1,950	2,700	1,300
	COPPER	60	40	60	55	65	45

TABLE 5.3 Variations in the concentration of lead, zinc and copper in the sediments of the Gleneedle and Glendhoo streams at different times during a period from February 1978 to October 1979. The sediment was collected from the edge of pools near stations 3 and 6.

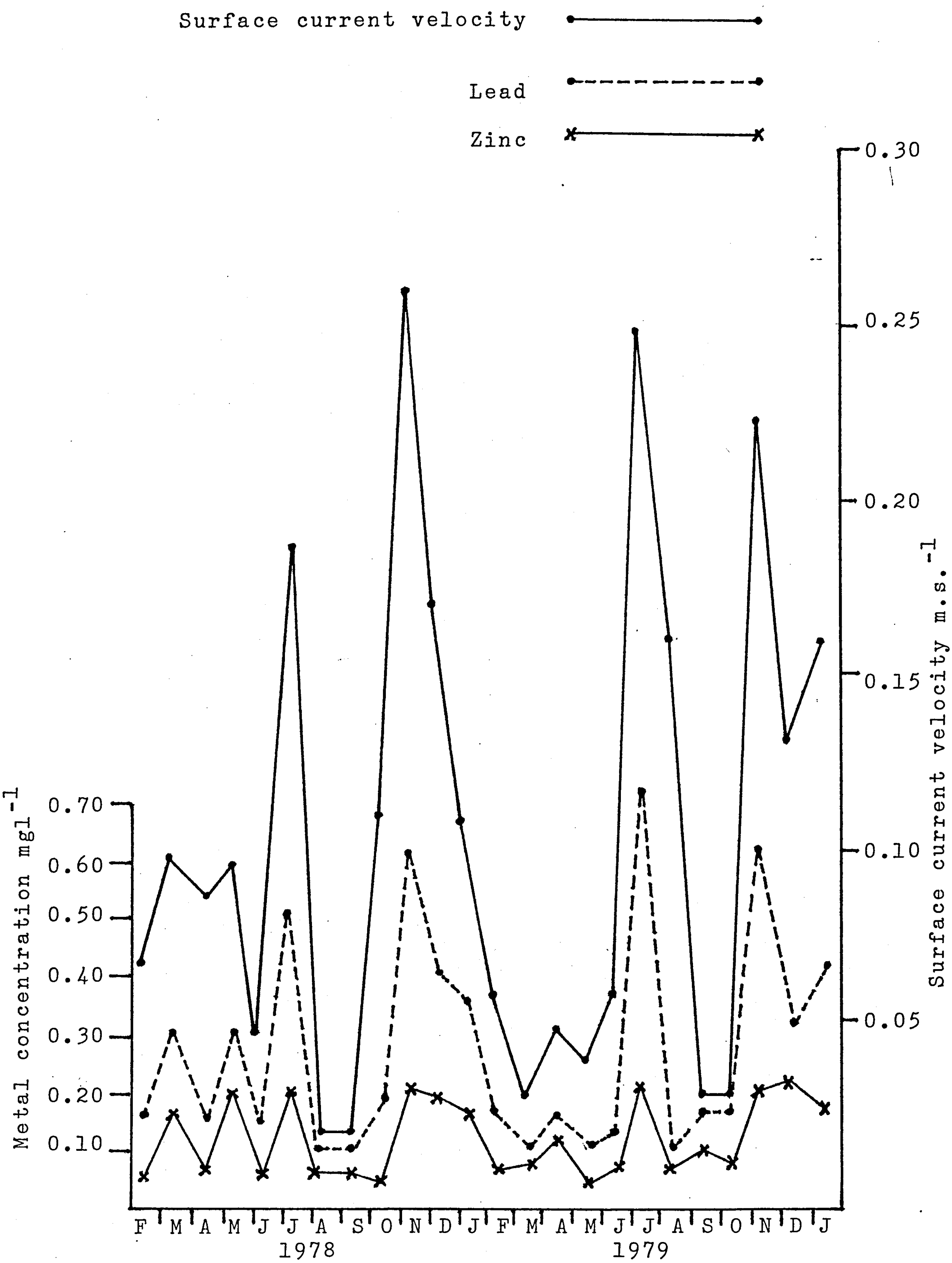


FIG. 5.3 Seasonal variations in the concentration of lead, zinc, and surface current velocity for the Glendhoo stream. Figures shown are the mean values of three sampling sites from February 1978 to January 1980.

of the equipment used, 0.01 μgl^{-1} for zinc and 0.02 μgl^{-1} for lead, even in conditions of heavy surface runoff.

The sediments of both streams were sampled on six occasions during the two year period and although it is not possible to draw firm conclusions from this sparse data (table 5.3), it does appear that metal concentrations were highest in the stream sediments during a period of minimum water flow, a direct contrast to the behaviour of the metals in solution.

The surface water velocity varied considerably throughout the sampling period, ranging from a minimum of 0.03 $m.s^{-1}$ during dry weather flow, to a maximum of 0.26$m.s^{-1}$ after heavy rain. The velocity, measured at three stations on each stream, gave consistently similar mean values for the two streams, on any one day.

Discussion

The concentrations of calcium and magnesium found in the Gleneedle and Glendhoo waters are very similar to those found by Hynes (1954)-table 5.4, for Manx streams with similar catchment areas and drainage characteristics; and although their waters are considered soft, they contain a slightly higher concentration of calcium, magnesium and sodium than Lake District rain, and Walla Brook (Arnold and Macan, 1969) a typical softwater Dartmoor stream (table 5.5.).

The greater sodium content of the Manx streams is likely to be due to the closer proximity of the sea, coupled with aerial transport and deposition of salt spray (Hynes, 1954) rather than intrusion by faecal matter or untreated sewage waste. Hynes (1954) records the sodium content of certain Manx streams, but the values are elevated due to the

STREAM	Ca^{++}	Mg^{++}
River Neb	4.0	3.0
Colby River	12.0	5.5
Silverburn	11.0	3.0
Santonburn	12.0	3.3
Crogga River	10.0	3.2
River Glass	7.0	3.0

TABLE 5.4. Details of water analysis in $mg.l^{-1}$ of certain Manx streams of similar catchment area to the Gleneedle and Glendhoo streams, (Data from Hynes, 1954)

WATER SOURCE	Ca^{++}	Mg^{++}	Na^{++}	NO_3-N
Walla Brook	1.14	0.77	5.70	0.07
Lake District rain	0.30	0.19	1.90	<0.02

TABLE 5.5. Ionic concentrations in $mg.l^{-1}$ for Walla Brook, a typical softwater Dartmoor stream, and Lake District rain, (After Arnold and Macan, 1969).

samples being taken at the mouth of the rivers with consequent intrusion by sea water.

Walla Brook is considerably richer in nitrate nitrogen than the Manx streams and this may be due to local agricultural run off; the area associated with the Manx streams is not cultivated or fertilized.

Manx rivers are generally free from industrial pollution owing to the absence of heavy industries on the Island. However, the presence of spoil heaps, the remains of mining activities on the Island, result in small but significant concentrations of heavy metal ions in the waters and sediments of certain streams (Pugh-Thomas, 1974).

The general aspect and physical conditions of the Gleneedle and Glendhoo streams are very similar and both streams drain the same valley. The major difference lies in their catchment areas, the Gleneedle stream rises in an area completely free from the influence of past mining activities, and the Glendhoo stream rises in an area previously subject to metal extraction, spoil heaps are common in this area of the valley and adits from disused mines drain into the Glendhoo stream.

During their productive period, ores of the metals lead, zinc and a small amount of copper were extracted from the mines (Mackay and Schnellman, 1963). The position of remaining spoil heaps suggest that, as found in rivers in Welsh mining areas (Carpenter, 1924; Abdullah and Royle, 1972, Brooker and Morris, 1980), ions of lead, zinc and possibly copper may be present in the waters of the Glendhoo stream.

Iron is often present in stream waters depending on the amount of soluble organic material which stabilizes the colloidal iron and enables it to remain in solution (Abdullah and Royle, 1972). Ferric iron is regarded by Jones (1964) as having a very low toxicity, whilst ferrous iron is likely to be precipitated as a red brown slime of ferric hydroxide often associated with the iron bacteria Leptothrix ochracea which forms a rust coloured layer on stones (Hynes, 1970a). There was no evidence of iron precipitation in either Manx stream. Doudoroff and Katz (1953) in their review of the literature of industrial waste, also list iron as less toxic than zinc, and Wood (1974) classifies metals in respect of their environmental pollution as (i) non critical (ii) toxic but insoluble (iii) toxic and accessible. Iron is placed in the first category by Wood with lead and zinc in the third group.

Clean water, in an area free from mineral influence, is described by Abdullah and Royle (1972), as having metal concentrations in the order of manganese 0.80, iron 1.7, nickel 0.50, copper 0.66, zinc 11.0, cadmium 0.41, and lead 0.70 μgl^{-1}. The concentrations of lead and zinc in the Gleneedle stream clearly fall within these limits. Wittman (1979) in his review of work in American rivers, says that the concentrations of lead and zinc in the streams prior to mining activities was in the range of 4 to 6 μgl^{-1}. The concentrations of lead and zinc in the Gleneedle water clearly falls within the range specified by Abdullah and Royle (1972) and Wittman (1979) so, using their criteria, the Gleneedle stream may be classified as clean.

It is interesting to compare the relative proportions of lead and zinc in the waters of certain Welsh rivers, with those of the Glendhoo stream. The Welsh River Rheidol is considered by Abdullah and Royle (1972) to have moderately high levels of zinc, 50 to 130 μgl^{-1}, and lead 1.3 to 2.4

$\mu g l^{-1}$, clearly the level of these metals in the Glendhoo stream, 0.05 to 0.20 mgl^{-1} zinc and 0.15 to 0.7 mgl^{-1} lead classify it as contaminated by the criteria of Abdullah and Royle. However, Brooker and Morris (1980) quote much higher levels of metals in the Rheidol, 0.012 to 0.336 mgl^{-1} zinc and 0.004 to 0.012 mgl^{-1} lead and attribute these figures to the Welsh Water Authority. Jones (1940) found concentrations of up to 1.2 mgl^{-1} zinc and 0.05 mgl^{-1} lead, and Brooker and Morris (1980) reported maxima of 2.002 mgl^{-1} zinc and 0.098 mgl^{-1} lead in the River Ystwyth.

Abdullah and Royle (1972) reported a greater abundance of zinc relative to lead in Welsh rivers, the ratio of zinc to lead being in the order of 45 for the Ystwyth, 54 for the Rheidol and 26 for the River Twymn. Brooker and Morris also found a greater concentration of zinc than lead in the Rheidol and Ystwyth. This is in direct contrast to the concentrations of those metals in the Manx stream where the conentration of zinc is consistently lower than that of lead, the ratio of zinc to lead being approximately 0.3 for the Glendhoo stream. A reverse relationship was found in the metal concentration of the sediments, here the concentration of zinc was greater than that of lead, the ratio of zinc to lead being in the order of 2.3 for the Glendhoo sediments, but Brown (1977) reported that the concentration of zinc was higher than copper in the waters of the River Hayle and lower than copper in the sediments. A similar general relationship was revealed by Aston and Thornton (1977) who found that the sediments of certain English rivers had a higher concentration of zinc than lead. Work on the transport of lead in lake Washington has shown that the metal is cycled through both the liquid and solid phases before entering the sediment

(Baier and Healy, 1977).

Periods of high surface current velocity in the Glendhoo stream were associated with periods of heavy rainfall, surface velocity having high values on two occasions during successive summers. The maximum concentration of metals in the Glendhoo stream occurred during periods of maximum surface current velocity. Brown (1977) reported that metal concentrations in the Hayle, a river in the mining district of Cornwall also increased during period of high flow. Grimshaw, Lewin and Fuge (1976) found a similar flushing effect in the rivers of the mining areas of Wales; at the onset of increasing run off there was a rise in the metal concentration of the rivers. Vivian and Massie (1977) also found that the zinc concentration in the River Tawe, South Wales was related to flow rate and ascribed this to leaching by surface run off. This flushing effect led Grimshaw, Lewin and Fuge (1977) to suggest that summer storms in mining areas may be significant for the stream biota. An explanation for this is given by Laurie and Jones (1938) who noted that lead and zinc were present in spoil heaps as the sulphides galena and blende. Galena, lead sulphide, and blende, zinc sulphide, both have limited solubility in water, but weathering slowly oxidizes them to sulphates which have a much greater solubility in water. During fine weather oxidation of the sulphides will proceed, and the product of oxidation, the sulphates, will remain in the spoil heap until leached out by heavy rain.

Previous workers (Carpenter, 1924 ; Abdullah and Royle 1972) have also suggested that the presence of heavy metals appears to impose severe constraints on the ecology of fresh-water streams, and Furmanska (1979) in a series of laboratory experiments demonstrated the harmful effects of copper and zinc on aquatic macrofauna.

The data presented in this chapter provide a prima facie case for the differences in the invertebrate fauna between the Gleneedle and Glendhoo streams being ascribed to the presence of lead and zinc in the waters of the Glendhoo stream. In this context the name 'Glendhoo stream' reflects its roots in Manx gaelic, translating to 'black stream'.

CHAPTER SIX

LABORATORY EXPERIMENTS

Introduction

Attempts were made to carry out field experiments by transferring mature larvae from the Gleneedle stream to the impoverished Glendhoo stream. Larvae were placed in weighted perforated containers, wedged between large stones, a technique also used by Humpesch and Elliott (1980). Unfortunately, these experiments were unsuccessful due to displacement of the containers by river turbulence, trampling by cattle or loss through other unknown causes.

Two series of laboratory experiments were conducted to observe the effect of placing (1) well grown larvae and (2) eggs, in water containing different concentrations of the ions of lead and zinc.

To prevent depletion of the populations of the small Gleneedle and Glendhoo streams, the insects used in the experiments were collected from suitable neighbouring sites. Eggs were obtained from gravid female imagines about to oviposit in the Gleneedle or Glendhoo stream.

Methods

Collection of Larvae

Preliminary investigation of the trichopteran fauna of similar waters within a two mile radius of the Gleneedle and Glendhoo streams revealed that the cased eruciform trichopteran fauna consisted exclusively of *Potamophylax latipennis*, and so well grown larvae of this species were collected from these waters, they had case lengths of 17 to 22mm.

Polycentropus flavomaculatus about 15mm long, Rhyacophila dorsalis 20mm to 22mm long and Protonemura meyeri 8mm to 10mm in length were collected from nearby streams, and Baetis rhodani nymphs 6mm to 8mm long were taken from the Gleneedle stream. All measurements being from the point of the labrum to the tip of the abdomen.

Collection of Eggs

The ovipositing habits of certain species of Ephemeroptera, together with methods for the collection of their eggs are well documented in the literature (Needham and Murphy, 1959; Elliott, 1972; Humpesch, 1979, 1980; Humpesch and Elliott, 1980) so only a brief description is given here.

Gravid female imagines were observed in their ovipositing flight, captured with a net, and transferred to a screw top specimen jar. Males of the same species were captured and retained in a similar manner. This procedure was carried out at different times for Potamophylax latipennis, Polycentropus flavomaculatus, Hydropsyche instabilis, Rhyacophila dorsalis, Protonemura meyeri and Baetis rhodani only one species being captured on each occasion.

For all adult insects, the abdomen of the female was crushed with tweezers in the laboratory, and the egg mass expelled into a watch glass. The tip of the male abdomen was also crushed, the sperm expelled, transferred to the egg mass and mixed with a preparation needle. The process of fertilization took about five minutes. After fertilization a small quantity of water from the Gleneedle stream was added to the watch glass and the contents transferred to a petri dish where the eggs were counted using a binocular microscope. This procedure eliminated parthenogenetic development by ensuring that the eggs were fertilized.

Identification of each species was confirmed by examining the remaining undamaged head parts, legs and wings of the adult insects with reference to standard keys (Hickin, 1967; Kimmins, 1972; Macan, 1973; and Hynes, 1977)

The ovipositing behaviour of one insect was unusual, *Potamophylax latipennis* was observed to deposit eggs directly onto the upper surface of overhanging streamside vegetation, the leaves of *Salix fragilis* being particularly favoured. The deposited egg mass was collected by vigorously washing the eggs off the leaf into a collecting dish.

Preparation of Stock Solutions

Standard solutions of 1000 $mgl.^{-1}$ were prepared by dissolving analar grade lead nitrate and zinc sulphate separately in distilled water and storing them in polythene containers.

Stock solutions were prepared by serial dilution of the standard solution with water taken from the Gleeneedle stream to produce solutions containing 0.15, 0.30 and 0.50 $mg.l^{-1}$ of lead and zinc respectively. The stock solution was made every two days to prevent loss of ions from the solution by adsorption.

Experimental Conditions for Larvae

Larvae were reared in plastic containers 25cm x 20cm x6cm deep, each container being divided into four compartments by a perforated plastic divider - fig. 6.1. The number of larvae distributed among the compartments is shown in table 6.1. The containers were filled to a depth of 5cm with stock solutions containing (i) 0.15, 0.30, 0.50 mgl^{-1} respectively, of zinc only.

SPECIES	TOTAL NUMBER OF LARVAE	NUMBER OF LARVAE IN EACH COMPARTMENT	NUMBER OF REPLICATES OF EACH SOLUTION
Protonemura meyeri	20	2	10
Baetis rhodani	50	5	10
Polycentropus flavomaculatus	10	1	10
Rhyacophila dorsalis	20	2	10
Hydropsyche instabilis	10	1	10
Potamophylax latipennis	20	2	10

TABLE 6.1. Number of larvae used in experiments on exposure to metal ions

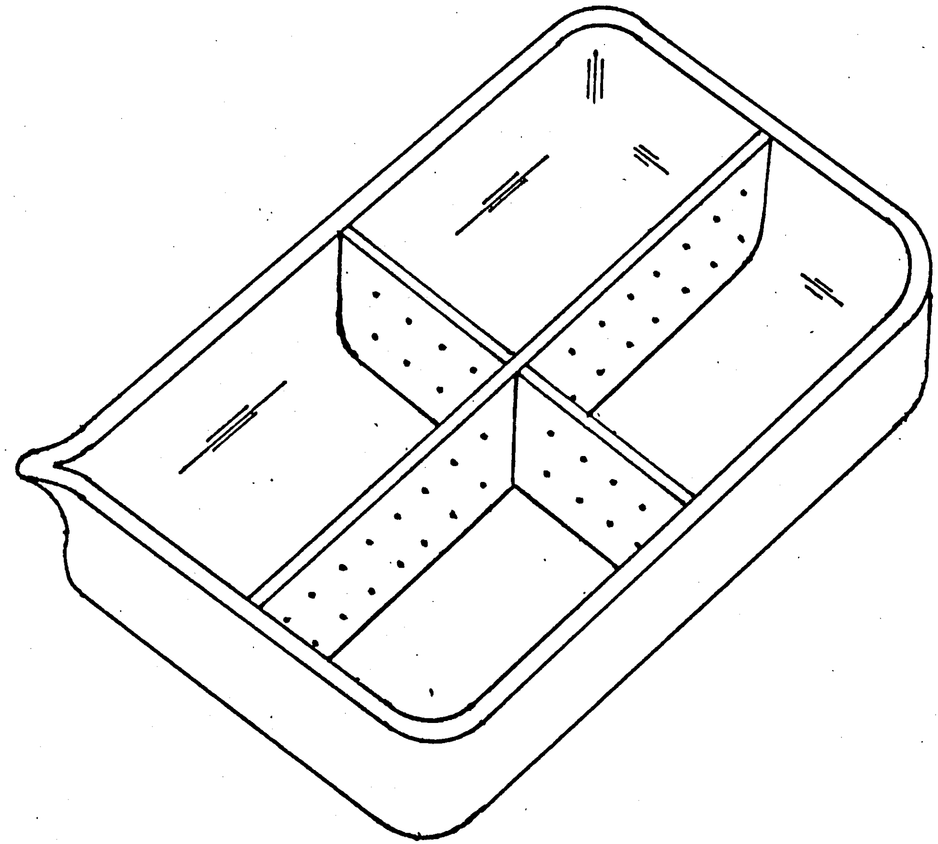


FIG. 6.1. Sketch of container used to rear larvae in solution containing metal ions.

(ii) 0.15, 0.30, 0.50 mgl^{-1} respectively, of lead only

(iii) 0.15, 0.30, 0.50 mgl^{-1} respectively of lead and zinc together.

In addition, containers were filled with water from the Gleneedle stream and also from the Glendhoo stream to which no additions of lead or zinc had been made. The container filled with water from the Gleneedle stream acted as a control.

Each container was continuously aerated and subject to natural light and temperature regimes since no attempt was made to regulate light or temperature.

The ionic concentration of lead and zinc in the stock test solutions was measured at the start and finish of each observation (see appendix tables A8 to A11) and the test solutions were renewed every two days to minimize loss of metal ions by adsorption or precipitation; Brown (1976) showed that metal levels remain constant over a 48 hour period. The larvae were examined every two days and exuvia removed when necessary. Suitable food was provided, as described under each species. All experiments were carried out in still water conditions (except that it was aerated), no attempt was made to provide an artificial current and death was assumed to have occurred for all species when the larvae no longer responded to tactile stimulation (Jones, 1964; Spehar, Anderson and Fiandt, 1978).

Experimental Conditions for Eggs

Fertilized eggs of _Protonemura meyeri_, _Baetis rhodani_, _Polycentropus flavomaculatus_, _Rhyacophila dorsalis_, _Hydropsyche instabilis_ and _Potamophylax latipennis_ were placed, according to species, in Petri dishes, three replicates being used for each species. The number of eggs used in each test varied from 23 to 184; the actual values are recorded in the appendix (tables A12 to A17).

After fertilization the dishes were filled to a depth of approximately 10mm with stock solutions containing (i) 0.15 $mg.l^{-1}$ lead (ii) 0.15 $mg.l^{-1}$ zinc (iii) 0.15 $mg.l^{-1}$ lead and 0.15 mgl^{-1} zinc. In addition dishes were also filled with water taken from the Glendhoo stream, water taken from the Gleneedle stream acted as a control; no additions of lead or zinc were made to the latter two sets of dishes.

The dishes were covered with their lids and placed on a laboratory bench in a small preparation room, the room was not subject to direct sunlight nor was the temperature of the room regulated, but it did not exceed 15°C. Water temperature in the dishes was not monitored but did not exceed that of the room. Humpesch (1980) suggested that temperature was not responsible for variations in the hatching success of Ecdyonurus spp. (Ephemeroptera). There was no forced aeration in the dishes and, as with the larval experiments, no attempt was made to regulate light or temperature, so each set of samples for one particular species was subject to the same constraints and variations in environmental parameters.

At the start and finish of each experiment the ionic concentrations of lead and zinc in the test solutions were measured. The test solutions were renewed every two days to minimise loss of metal ions (Brown, 1976), the temperature of the replacement stock solution was allowed to equilibrate with that of the room, and prior to use was saturated with oxygen by aeration.

The eggs were examined daily, as near as possible to 10.00a.m. using a binocular microscope and the hatching time, in days, was recorded. When hatching commenced, the newly emerged larvae were removed and counted, the count being expressed as a cumulative percentage.

A total of ninety individual experiments were carried out over a period ranging from 12th May, 1979 to 19th November, 1979, with approximately monthly intervals between the start of observations on each of the six species.

Results of Experiments with Larvae

Protonemura meyeri

P. meyeri is known to feed on periphytic and epilithic material (Jones, 1950; Macan, 1963; Hynes, 1977) and each compartment containing these nymphs was provided with two small stones taken from the Gleneedle stream, the stones were changed every five days. The effects of lead and zinc in the environment of P.meyeri are shown in table 6.2. 70% of the nymphs survived in the control solution with 80% of them successfully completing one moult. The presence of 0.15, 0.30 and 0.50 mgl^{-1} lead reduced the survival to 45%, 20% and 5% respectively, the figures, for zinc were very similar. The effects of the two metals acting together were invariably more toxic than either metal acting on its own. Only 15% of numphs survived more than 10 days and none survived more than 30 days in a solution containing 0.5 mgl^{-1} lead and 0.5 mgl^{-1} zinc. The nymphs appeared to be most vulnerable during ecdysis, 80% survived a moult in the control solution compared with 20% that survived a moult in the combined presence of 0.3 mgl^{-1} lead and 0.3 mgl^{-1} zinc (see also appendix table A9). Because the larvae were collected from the streams at unknown stages of the intermoult phase, it is not possible to draw firm conclusions about the maximum period before a successful moult (a moult was regarded as successful if the nymph survived for a minimum period of one day after ecdysis). However, the data presented in table 6.2. do suggest that the period before successful moulting decreased slightly with increased concentrations of metal ions, and that the effects of lead and zinc acting together tended to be greater than that of each metal acting singly.

Baetis rhodani

The compartments containing nymphs of *B. rhodani* were provided with two stones, taken from the Gleneedle stream, every five days to provide a source of food for this epilithic feeder (Jones, 1950). *B. rhodani* appeared to be the most sensitive of the nymphs tested to the presence of lead and zinc, see table 6.3. only 4% successfully survived a moult in 0.15 mgl^{-1} zinc and 6% moulted successfully in 0.15 mgl^{-1} lead. Only 4% of the nymphs survived longer than 10 days under the combined influence of 0.3 mgl^{-1} lead and 0.3mgl^{-1} zinc.

Polycentropus flavomaculatus

To facilitate net attachment, two small stones taken from the Gleneedle stream were provided, net spinning was accomplished within 12 hours of the larvae being placed in the container. Food was supplied by placing a small dipteran larva in the net every two days. Of the species examined, *P. flavomaculatus* exhibited the greatest resistance towards the effects of lead and zinc, as shown in table 6.4. 70% of the larvae survived for over 40 days in the combined presence of 0.5 mgl^{-1} lead and 0.5 mgl^{-1} zinc, compared with a survival rate of 90% for larvae incubated in water from the Glendhoo stream and 90% for those incubated in the control solution.

None of the larvae pupated, and it was not possible to draw firm conclusions on the moulting success because the net of P. flavomaculatus obscured observations.

Rhyacophila dorsalis

The predatory free ranging trichopteran R. dorsalis normally locates its prey by tactile stimulation (Moss, 1980), but under laboratory conditions failed to capture the dipteran larva provided as food. R. dorsalis failed to develop in any of the test solutions or in the control solution so it is not possible to say if this failure is due to the presence of metal ions.

Hydropsyche instabilis

Under the constraints of still water conditions the larvae of H. instabilis failed to construct a net, a feature also reported by Hynes (1970 a), and Philipson and Moorhouse (1974). H. instabilis failed to develop in any of the test solutions or in the control solutions and did not survive for more than three days. It is not possible to say if the failure to develop was connected with the presence of metal ions or if it was always due to environmental factors.

Potamophylax latipennis

The mineral cased P. latipennis, a large particle shredder, feeding on allochthonous material (Moss, 1980) was provided with partly decomposed willow leaves (Salix fragilis) or with willow leaves that had been soaked in Gleneedle stream water for a minimum period of two weeks (Moss, 1980), the leaves were collected from the Gleneedle stream side. This procedure eliminated the possibility of metal uptake by the larvae from the food source. The pupation success and condition of the emergent teneral adults are recorded in figs. 6.2. to figs. 6.4. and table 6.5.

In the control solution all the larvae of P. latipennis successfully entered the pupal stage and the addition of 0.15 mgl^{-1} lead and 0.15 mgl^{-1} zinc acting singly or in combination appeared to have no effect on larvae entering pupation. There was however a small reduction in the numbers of larvae pupating as the metal concentrations increased, the effects of lead and zinc acting together were greater than the effect of either element acting on its own. The addition of 0.5 mgl^{-1} lead to the incubation medium reduced the number of P. latipennis larvae pupating, from 20 (100%) in the control solution to 15 (75%): an identical effect was obtained with the same concentration of zinc. When combined in these proportions the effect of the two metals was increased and the number of larvae that entered the pupal stage was reduced to 12 (60%).

The presence of lead and zinc also affected the pupation success, measured as the number emerging, of P. latipennis. The effects of the metals acting singly were very similar, 0.15 mgl^{-1} lead reduced pupal emergence to 95% (19) when compared to 100% (20) emergence in the control solution. The addition of 0.15 mgl^{-1} zinc reduced emergence to 90% (18) and the presence of 0.5 mgl^{-1} zinc reduced pupal success by 35%, but pupal success was reduced by 40% in the presence of 0.5 mgl^{-1} lead, only 12 teneral adults emerged. The most damaging effects occurred when these metals acted together, in the combined presence of 0.5 mgl^{-1} lead and 0.5 mgl^{-1} zinc emergence from the pupal stage was completely inhibited.

The existence of lead and zinc in the incubation medium also affected the time spent in pupation. The mean pupation

INCUBATION MEDIUM	PERCENTAGE ALIVE AFTER				PERCENTAGE SUCCESSFULLY MOULTING	MAXIMUM PERIOD IN DAYS BEFORE A SUCCESSFUL MOULT
	2 DAYS	5 DAYS	10 DAYS	30 DAYS		
Gleneedle stream water plus 0.15 mgl^{-1} zinc	85 (17)	80 (16)	70 (14)	45 (9)	70 (14)	13
Gleneedle stream water plus 0.15 mgl^{-1} lead	100 (20)	85 (17)	80 (16)	45 (9)	80 (16)	13
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead.	75 (15)	70 (14)	55 (11)	20 (4)	55 (11)	12
Gleneedle stream water plus 0.30 mgl^{-1} zinc.	80 (16)	70 (14)	55 (11)	20 (4)	30 (6)	12
Gleneedle stream water plus 0.30 mgl^{-1} lead.	85 (17)	80 (16)	55 (11)	20 (4)	30 (6)	11
Gleneedle stream water plus 0.30 mgl^{-1} zinc and 0.30 mgl^{-1} lead.	55 (11)	45 (9)	30 (6)	10 (2)	20 (4)	9
Gleneedle stream water plus 0.50 mgl^{-1} zinc.	65 (13)	55 (11)	40 (8)	5 (1)	15 (3)	10
Gleneedle stream water plus 0.50 mgl^{-1} lead	70 (14)	60 (12)	30 (6)	5 (1)	15 (3)	11
Gleneedle stream water Plus 0.50 mgl^{-1} zinc and 0.50 mgl^{-1} lead.	45 (9)	30 (6)	15 (3)	0	0	0
Glendhoo stream water only	90 (18)	80 (16)	50 (10)	25 (5)	60 (12)	12
CONTROL Gleneedle stream water only	100 (20)	90 (18)	85 (17)	70 (14)	80 (16)	14

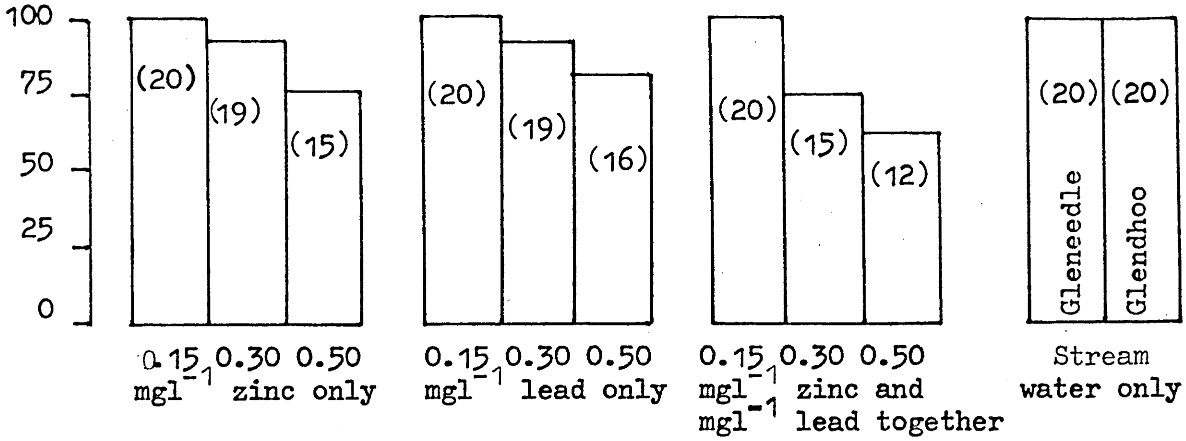
TABLE 6.2. **Observations of nymphs of <u>Protonemura meyeri</u> placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc. Figures in brackets refer to numbers.**

INCUBATION MEDIUM	PERCENTAGE ALIVE AFTER 2 DAYS	5 DAYS	10 DAYS	20 DAYS	PERCENTAGE SUCCESSFULLY MOULTING	MAXIMUM PERIOD IN DAYS BEFORE 1ST SUCCESSFUL MOULT
GLENEEDLE STREAM WATER PLUS 0.15 $mg.l^{-1}$ ZINC	94 (47)	66 (33)	58 (29)	0	4 (2)	13
GLENEEDLE STREAM WATER PLUS 0.15 $mg.l^{-1}$ LEAD	92 (46)	62 (31)	58 (29)	0	6 (3)	13
GLENEEDLE STREAM WATER PLUS 0.15 $mg.l^{-1}$ ZINC PLUS 0.15 $mg.l^{-1}$ LEAD	58 (29)	44 (22)	8 (4)	0	0	-
GLENEEDLE STREAM WATER PLUS 0.30 $mg.l^{-1}$ ZINC	90 (45)	48 (24)	8 (4)	0	0	-
GLENEEDLE STREAM WATER PLUS 0.30 $mg.l^{-1}$ LEAD	90 (45)	52 (26)	12 (6)	0	0	-
GLENEEDLE STREAM WATER PLUS 0.30 $mg.l^{-1}$ ZINC PLUS 0.30 $mg.l^{-1}$ LEAD	32 (16)	18 (9)	4(2)	0	0	-
GLENEEDLE STREAM WATER PLUS 0.50 $mg.l^{-1}$ ZINC	42 (21)	0	0	0	0	-
GLENEEDLE STREAM WATER PLUS).50 $mg.l^{-1}$ LEAD	36 (18)	0	0	0	0	-
GLENEEDLE STREAM WATER PLUS 0.50 $mg.l^{-1}$ ZINC PLUS 0.50 $mg.l^{-1}$ LEAD	14 (7)	0	0	0	0	-
GLENDHOO STREAM WATER ONLY	96 (48)	78 (39)	68 (34)	6(3)	32 (16)	12
CONTROL GLENEEDLE STREAM WATER ONLY	98 (49)	80 (40)	72 (36)	58(29)	80 (40)	15

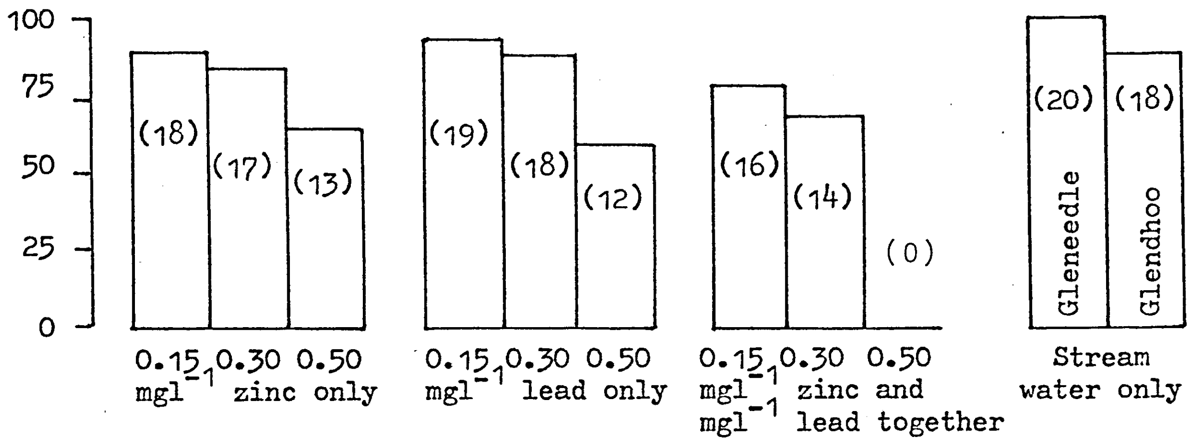
TABLE 6.3. Observations of nymphs of Baetis rhodani placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc in the laboratory. Figures in brackets refer to numbers.

INCUBATION MEDIUM	PERCENTAGE ALIVE AFTER			
	5 DAYS	10 DAYS	20 DAYS	40 DAYS
GLENEEDLE STREAM WATER PLUS 0.15 $mg.l^{-1}$ ZINC	100 (10)	100 (10)	100 (10)	100 (10)
GLENEEDLE STREAM WATER PLUS 0.15 $mg.l^{-1}$ LEAD	100 (10)	100 (10)	100 (10)	100 (10)
GLENEEDLE STREAM WATER PLUS 0.15 $mg.l^{-1}$ ZINC AND 0.15 $mg.l^{-1}$ LEAD	100 (10)	100 (10)	90 (9)	80 (8)
GLENEEDLE STREAM WATER PLUS 0.30 $mg.l^{-1}$ ZINC	100 (10)	100 (10)	100 (10)	100 (10)
GLENEEDLE STREAM WATER PLUS 0.30 $mg.l^{-1}$ LEAD	100 (10)	100 (10)	90 (9)	90 (9)
GLENEEDLE STREAM WATER PLUS 0.30 $mg.l^{-1}$ ZINC AND .30 $mg.l^{-1}$ LEAD	100 (10)	90 (9)	90 (9)	90 (9)
GLENEEDLE STREAM WATER PLUS 0.50 $mg.l^{-1}$ ZINC	100 (10)	90 (9)	80 (8)	80 (8)
GLENEEDLE STREAM WATER PLUS 0.50 $mg.l^{-1}$ LEAD	100 (10)	100 (10)	90 (9)	80 (8)
GLENEEDLE STREAM WATER PLUS 0.50 $mg.l^{-1}$ ZINC AND 0.50 $mg.l^{-1}$ LEAD	100 (10)	100 (10)	90 (9)	70 (7)
GLENDHOO STREAM WATER ONLY	100 (10)	100 (10)	90 (9)	90 (9)
CONTROL GLENEEDLE STREAM WATER ONLY	100 (10)	100 (10)	100 (10)	90 (9)

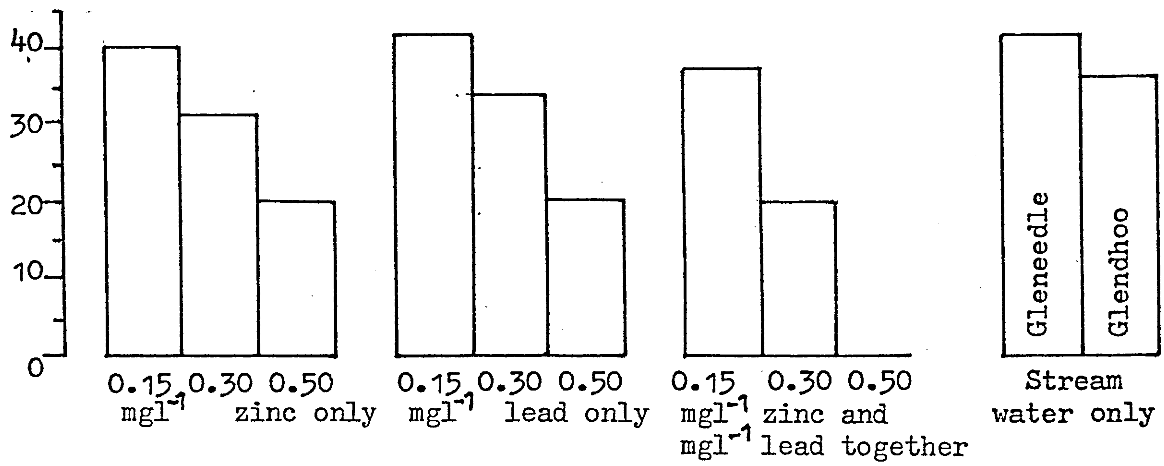
TABLE 6.4 Observations of larvae of Polycentropus flavomaculatus placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc. Figures in brackets refer to numbers.



(a) Percentage of larvae pupating



(b) Percentage of teneral adults emerging from pupation

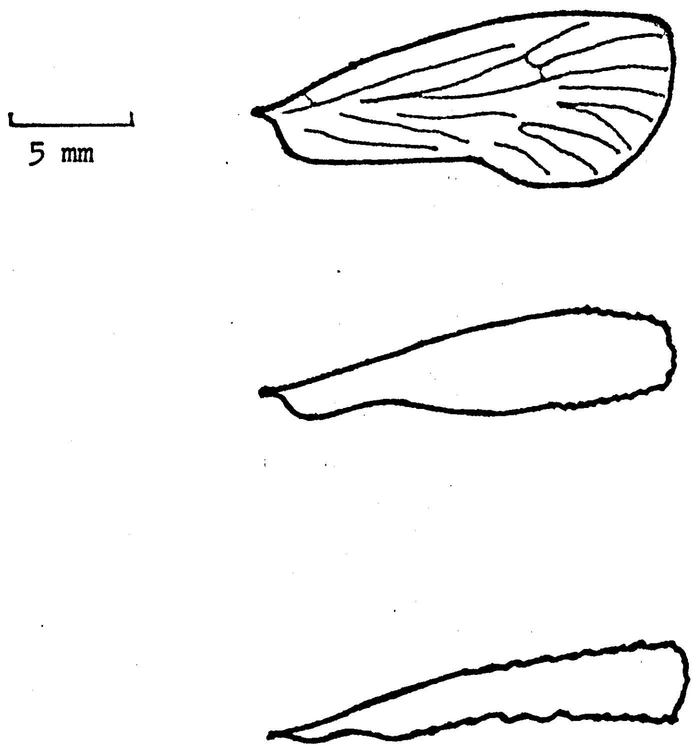


(c) Mean number of days spent in pupation

FIG. 6.2. Observations of well grown Potamophylax latipennis larvae placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc in the laboratory. Each test comprised five samples of four larvae. Figures in brackets refer to numbers of survivors.

INCUBATION MEDIUM	CONDITION OF TENERAL ADULT ON EMERGENCE
Gleneedle stream water plus 0.15 mgl^{-1} zinc	15 teneral adults emerged apparently normal and capable of flight. 3 teneral adults emerged with under-developed wings incapable of being fully opened.
Gleneedle stream water plus 0.15 mgl^{-1} lead	15 teneral adults emerged apparently normal and capable of flight. 4 teneral adults emerged with under-developed wings incapable of being fully opened.
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead.	All teneral adults emerged with underdeveloped wings incapable of being fully opened. The insects were active but incapable of flight.
Gleneedle stream water plus 0.30 mgl^{-1} zinc.	All teneral adults emerged with underdeveloped wings incapable of being fully opened. The insects were active but unable to fly.
Gleneedle stream water plus 0.3 mgl^{-1} lead.	All teneral adults emerged with underdeveloped wings incapable of being fully opened. The insects were active but unable to fly.
Gleneedle stream water plus 0.30 mgl^{-1} zinc and 0.30 mgl^{-1} lead.	All teneral adults emerged with grossly distorted wings, were active but unable to fly.
Gleneedle stream water plus 0.50 mgl^{-1} zinc.	All teneral adults emerged with grossly distorted wings, were active but incapable of flight.
Gleneedle stream water plus 0.50 mgl^{-1} lead.	All teneral adults emerged with grossly distorted wings were active but incapable of flight.
Gleneedle stream water plus 0.50 mgl^{-1} zinc and 0.50 mgl^{-1} lead.	Teneral adults did not emerge from pupation. Observations were discontinued after 50 days.
Glendhoo stream water only.	All teneral adults emerged with underdeveloped wings that were incapable of being fully extended. The insects were active but unable to fly.
CONTROL Gleneedle stream water only.	All emergent teneral adults appeared normal and were capable of flight.

TABLE 6.5. Observations on the condition of emergent teneral adults of <u>Potamophylax latipennis</u> pupated in water from the Gleneedle stream to which had been added different concentrations of lead and zinc.



(a) Normal Wing:

Typical outline of the wing of a teneral adult incubated in the control solution of Gleneedle stream water only.

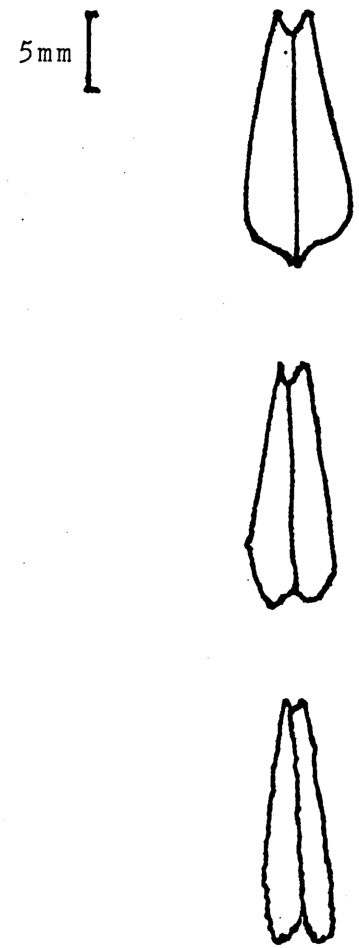
(b) Underdeveloped Wing:

Typical outline of the wing of a teneral adult incubated in the medium containing 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead.

(c) Grossly Distored Wing:

Typical outline of the wing of a teneral adult incubated in the medium containing 0.3 mgl^{-1} zinc and 0.3 mgl^{-1} lead.

FIG. 6.3. Outline of the forewings of teneral adults of <u>Potamophylax latipennis</u> incubated in Gleneedle stream water containing different concentrations of lead and zinc.



(a) Normal Wings: Incubated in the control solution of Gleneedle stream water only.

(b) Underdeveloped Wings: Incubated in medium containing 0.5mgl^{-1} zinc and 0.15 mgl^{-1} lead.

(c) Grossly distorted Wings: Incubated in medium containing 0.30mgl^{-1} zinc and 0.3 mgl^{-1} lead.

FIG. 6.4. Dorsal views of the folded wings of teneral adults of *Potamophylax latipennis* incubated in Gleneedle stream water containing different concentrations of lead and zinc.

time of 42 days in the control solution was reduced to 38 days under the combined influence of 0.15 mgl^{-1} lead and 0.15 mgl^{-1} zinc, and to 20 days under the single influence of 0.5 mgl^{-1} lead or 0.5 mgl^{-1} zinc.

The condition of the teneral adults on emergence was considerably influenced by the amount of metal present in the incubation media, see figs. 6.2. and 6.3. Larvae pupated in the control solution appeared normal on emergence, and after casting off the pupal integument were capable of flight. Of the larvae pupated in 0.15 mgl^{-1} zinc 90% emerged (18 teneral adults), but of these 15% (3 teneral adults) were incapable of fully extending their wings, the remaining 70% were apparently normal and able to fly. 95% (19 teneral adults) emerged from the test solution containing 0.15 mgl^{-1} lead and 4 of these were incapable of flight due to the insects inability to extend their wings. The combined effects of 0.15 mgl^{-1} lead and 0.15 mgl^{-1} zinc was to cause all insects to emerge incapable of extending their wings. As the concentration of lead and zinc increased in the incubation media so did the extent of wing malformation, but in all instances the emerged teneral adults were active, able to run about, but incapable of flight. Concentrations of 0.5 mgl^{-1} lead or 0.5 mgl^{-1} zinc acting singly produced emergent insects with wing development so inhibited that they appeared as crumpled tubes. The wings of all insects of this species pupated in water from the Glendhoo stream were also underdeveloped, the adults being active but incapable of flight.

Results of Experiments with Eggs

Protonemura meyeri

The hatching success of P. meyeri is shown in table 6.6., 80% of the eggs hatched within a period of 60 days in the control solution, the mean hatching time being 50 days. The addition of 0.15 mgl^{-1} lead or 0.15 mgl^{-1} zinc, singly, reduced the mean hatching time to 35 days with a dramatic reduction of hatching success to 5%. Eggs placed in water taken from the Glendhoo stream, or placed in the test solution containing 0.15 mgl^{-1} lead together with 0.15 mgl^{-1} zinc failed to hatch after a 60 day period when observations ceased.

Baetis rhodani

Eggs of B. rhodani had a cumulative hatching success of 91% after 65 days incubation, with a mean hatching time of 46 days, in the control solution. This compares with records made by Elliott (1972) of a 94% hatching success for eggs of B. rhodani in the laboratory. Bohle (1969) also records that 50% of the nymphs of B. rhodani took 43 days to hatch at a temperature in the region of 7°C whilst Humpesch (1980) found the 50% of artificially fertilized eggs of Ecdyonurus dispar (Ephemeroptera) took between 53 and 57 days to hatch at a constant temperature of 10°C in the laboratory.

The effect of 0.15 mgl^{-1} lead or 0.15 mgl^{-1} zinc acting singly in the incubation medium was to reduce the mean hatching time to 26 days and 29 days respectively with a corresponding reduction in hatching success to 2% and 4%, as shown in table 6.7. The effects of 0.15 mgl^{-1} lead acting in combination with 0.15 mgl^{-1} zinc was to prevent hatching completely during the 60 day observation period, an effect also noted with eggs incubated in water from the Glendhoo stream.

INCUBATION MEDIUM	NUMBER OF REPLICATE SAMPLES	NUMBER OF EGGS		MEAN TIME IN DAYS TO FIRST HATCHING	MEAN CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
		MEAN	RANGE		40 DAYS	45 DAYS	50 DAYS	60 DAYS
Gleneedle stream water plus 0.15 mgl^{-1} zinc	3	99	72 - 136	35	5	5	5	5
Gleneedle stream water plus 0.15 mgl^{-1} lead	3	101	87 - 121	35	4	5	5	5
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead	3	99	62 - 145	-	0	0	0	0
Glendhoo stream water only	3	87	70 - 105	-	0	0	0	0
CONTROL Gleneedle stream water only	3	85	76 - 95	50	0	5	65	80

TABLE 6.6. Observations of the hatching success of eggs of Protonemura meyeri fertilized on 12th May, 1979, and placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc in the laboratory.

INCUBATION MEDIUM	NUMBER OF REPLICATE SAMPLES	NUMBER OF EGGS		MEAN TIME IN DAYS TO FIRST HATCHING	MEAN CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
		MEAN	RANGE		50 DAYS	55 DAYS	60 DAYS	65 DAYS
Gleneedle stream water plus 0.15 mgl^{-1} zinc	3	137	121 - 151	29	3	3	4	4
Gleneedle stream water plus 0.15 mgl^{-1} lead	3	145	103 - 194	26	2	2	2	3
Gleneedle stream water plus 0.15^{-1} zinc and 0.15 mgl^{-1} lead	3	121	98 - 146	-	0	0	0	0
Glendhoo stream water only	3	138	121 - 157	-	0	0	0	0
CONTROL Gleneedle stream water only	3	120	99 - 142	46	12	53	91	91

TABLE 6.7. Observations of the hatching success of eggs of Baetis *rhodani* fertilized on 2nd August, 1979, and placed in water from the Gleneedle stream to which had been added different concnetrations of lead and zinc in the laboratory.

INCUBATION MEDIUM	NUMBER OF REPLICATE SAMPLES	NUMBER OF EGGS		MEAN TIME IN DAYS TO FIRST HATCHING	MEAN CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
		MEAN	RANGE.		55 DAYS	60 DAYS	65 DAYS	70 DAYS
Gleneedle stream water plus 0.15 mgl^{-1} zinc	3	54	50 - 58	21	2	4	5	5
Gleneedle stream water plus 0.15 mgl^{-1} lead	3	75	61 - 94	24	1	4	6	6
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead	3	40	24 - 49	-	0	0	0	0
Glendhoo stream water only	3	53	36 - 72	-	0	0	0	0
CONTROL Gleneedle stream water only	3	43	32 - 56	51	15	41	89	89

TABLE 6.8. Observations of the hatching success of eggs of Polycentropus flavomaculatus fertilized on 5th August, 1979, and placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc in the laboratory.

INCUBATION MEDIUM	NUMBER OF REPLICATE SAMPLES	NUMBER OF EGGS		MEAN TIME IN DAYS TO FIRST HATCHING	MEAN CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
		MEAN	RANGE		35 DAYS	40 DAYS	45 DAYS	50 DAYS
Gleneedle stream water plus 0.15 mgl^{-1} zinc.	3	31	23 - 42	19	2	4	8	10
Gleneedle stream water plus 0.15 mgl^{-1} lead	3	89	56 - 121	21	2	4	8	10
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead	3	33	18 - 49	-	0	0	0	0
Glendhoo stream water only	3	81	60 - 100	-	0	0	0	0
CONTROL Gleneedle stream water only	3	67	40 - 90	29	8	53	69	69

TABLE 6.9. Observations of the hatching success of eggs of *Rhyacophila dorsalis* fertilized on 20th July, 1979, and placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc in the laboratory.

INCUBATION MEDIUM	NUMBER OF REPLICATE SAMPLES	NUMBER OF EGGS		MEAN TIME IN DAYS TO FIRST HATCHING	MEAN CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
		MEAN	RANGE		40 DAYS	45 DAYS	50 DAYS	60 DAYS
Gleneedle stream water plus 0.15 mgl^{-1} zinc	3	148	110 - 184	24	2	4	4	9
Gleneedle stream water plus 0.15 mgl^{-1} lead.	3	128	97 - 164	21	3	5	6	9
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead	3	118	105 - 134	-	0	0	0	0
Glendhoo stream water only	3	102	76 - 119	-	0	0	0	0
CONTROL Gleneedle stream water	3	125	121 - 130	38	5	48	84	84

TABLE 6.10 Observations of the hatching success of eggs of Hydropsyche instabilis fertilized on 15th June, 1979, and placed in water from the Gleneedle stream to which had been added different concentrations of lead and zinc in the laboratory.

INCUBATION MEDIUM	NUMBER OF REPLICATE SAMPLES	NUMBER OF EGGS		MEAN TIME IN DAYS TO FIRST HATCHING	MEAN CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
		MEAN	RANGE		20 DAYS	25 DAYS	30 DAYS	35 DAYS
Gleneedle stream water plus 0.15 mgl^{-1} zinc	3	114	94 - 136	18	8	15	20	20
Gleneedle stream water plus 0.15 mgl^{-1} lead.	3	99	80 - 120	17	9	20	23	23
Gleneedle stream water plus 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead.	3	107	101 - 112	-	0	0	0	0
Glendhoo stream water only	3	109	88 - 121	-	0	0	0	0
CONTROL Gleneedle stream water only	3	112	94 - 136	19	12	73	94	94

TABLE 6.11 Observations of the hatching success of eggs of <u>Potamophylax latipennis</u> fertilized on 6th September, 1979, and placed in water from the Gleneedle stream to which had been added different concentration of lead and zinc in the laboratory.

Polycentropus flavomaculatus

Eggs of P.flavomaculatus incubated in the control solution had a mean hatching time of 51 days with a cumulative hatching success of 89% over a period of 65 days, as shown in table 6.8. The presence of 0.15 mgl^{-1} zinc or 0.15 mgl^{-1} lead acting singly in the test solutions reduced the mean hatching time to 21 days and 24 days respectively, with the corresponding mean cumulative hatching success similarly reduced to 5 % and 6 % . The combined effects of 0.15 mgl^{-1} lead and 0.15 mgl^{-1} zinc were similar to those of eggs incubated in water from the Glendhoo stream in each case the eggs had failed to hatch after a period of 70 days when observations ceased.

Rhyacophila dorsalis

The effect of metal ions on the hatching success of R. dorsalis is listed in table 6.9. The mean hatching time of 29 days in the control solution was reduced to 21 days under the influence of 0.15 mgl^{-1} lead, and to 19 days in the presence of 0.15 mgl^{-1} zinc. Reductions in the hatching success were also apparent, 0.15 mgl^{-1} zinc or 0.15 mgl^{-1} lead acted independently, to dramatically reduce hatching success to 10%, compared with 70%, for the control solution, and hatching failed to occur in both the Glendhoo stream water and in the test solution containing 0.15 mgl^{-1} lead together with 0.15 mgl^{-1} zinc.

Hydropsyche instabilis

Observations of the effects of lead and zinc on the eggs of H.instabilis, recorded in table 6.10. show similar general effects to the results obtained with eggs of the other species.

The presence of either 0.15 mgl^{-1} lead or 0.15 mgl^{-1} zinc dramatically reduced the cumulative hatching success from 84% in the control solution to 9% in the test solutions. The mean hatching time was also reduced, from 38 days under control conditions to 24 days under the influence of 0.15 mgl^{-1} zinc and 21 days when exposed to 0.15 mgl^{-1} lead. When these metals were present together, hatching was completely inhibited, as was hatching in water taken from the Glendhoo stream..

Potamophylax latipennis

The mean hatching time for P. latipennis in the control solution of Gleneedle water was 19 days, with a variance of 0.2; additions of 0.15 mgl^{-1} zinc to the solution reduced the mean hatching time to 18 days with a variance of 0.8, as shown in table 6.11. When 0.15 mgl^{-1} lead was present the mean hatching time was similarly reduced to 18 days but with a variance of 0.6, and the effect of 0.15 mgl^{-1} lead acting together with 0.15 mgl^{-1} zinc was to prevent hatching altogether; an effect also observed with eggs incubated in water taken from the Glendhoo stream which similarly failed to hatch. The addition of metal ions reduced the mean cumulative hatching, success from 94% in the control solution to 23% in the presence of 0.15 mgl^{-1} lead and to 20% when 0.15 mgl^{-1} zinc was present.

The mean hatching time for replicate observations was remarkably consistent for each species, the standard error varying from 0.41 for Rhyacophila dorsalis to 0.81 for Protonemura meyeri.

Discussion of Experimental Results

Owing to the lack of success with field experiments, any conclusions about the possible effects of lead or zinc ions must be based on the laboratory experiments, and these could give very different results from whole natural environments. Kaminski (1980) says "laboratory biological test analysis of water samples cannot reveal a number of interactions between toxicants and elements of the ecosystem". Brown, Shurben and Shaw (1970) also found that the toxic effects of metals observed in the field are always greater than those obtained by adding the possible contributions of each heavy metal known to be present.

Observations indicate that nymphs of *Baetis rhodani* *Protonemura meyeri* are tolerant of the combined effects of lead and zinc in the order of 0.15 mgl^{-1}, but only between moults, mortality occuring at or during ecdysis; Clubb, Gaufin, and Lords (1975) also found that the presence of cadmium inhibited the moulting and successful emergence of the plecopterans *Pteronarcys californica* and *Arcynopteryx signata* in the laboratory. Lockwood and Inman (1973) demonstrated that cuticle permeability of the amphipod *Gammarus duebeni* varied according to the moult and suggested that this must have been responsible for increased metal absorption. A similar conclusion was reached by Anderson (1950) who observed that *Daphnia magna* were most sensitive to heavy metals at the moulting stage. Anderson (1950) also suggested that this might apply to arthropods generally. Yager and Harry (1964) concluded that metal ions were effective in disrupting the membrane permeability of the Planorbid snail *Taphius glabratus*. A similar mechanism may be operating in *Baetis tenax* and *Protonemura meyeri*. However, Spehar, Anderson and Fiandt (1978) observed that lead concentrations as high as 565 μgl^{-1} did not cause a significant decrease in the stonefly (Plecoptera) Pteronarcys dorsata after 25 days of exposure and Clubb et al (1975) found that Pteronarcys californica was relatively insensitive to the presence of cadmium. Spehar etal concluded that some aquatic invertebrates could be affected by lower concentrations of the metal when exposed over a longer period.

Laboratory experiments indicate that the net spinning trichopteran Polycentropus flavomaculatus is resistant in the larval stage to the presence of lead and zinc, in amounts greater than the annual mean levels found in the Glendhoo stream. The mean number of larvae surviving for a given period was the same for those larvae in the control solution as it was for those placed in water from the Glendhoo stream. Increasing the combined amounts of lead and zinc to 0.5 mgl^{-1} reduced the number of P. flavomaculatus larvae surviving for this period by 30%. Jones (1940) reports that the sum total of 3 larvae of P. flavomaculatus were collected on 5 different sampling occasions in the River Ystwyth polluted with 0.05 mgl^{-1} lead and 0.9 mgl^{-1} zinc.

The presence of lead and zinc in the environment of Potamophylax latipennis caused a reduction in the number of teneral adults that emerged from pupation; those teneral adults that did emerge had spent less time in pupation than those incubated in the control solution, and emerged with under-developed wings. A similar effect was noted by Laurie and Jones (1938) who found that larvae of Dytiscus marginalis pupated but failed to metamorphose successfully in a solution containing 20 mgl^{-1} lead. It is possible that the teneral adults of Potamophylax latipennis that emerged in a shorter time, were less mature, so that their underdeveloped wings were equivalent to a state of development several days before normal emergence. In all cases the effects of lead and zinc in combination were greater than either metal on its own. A similar illustration of the synergistic toxic effects of heavy metals is reported by Lloyd (1961) when studying the effects of copper and zinc on trout, and by Bandt (1946) when working with roach and trout. Furmanska (1979) reports that the combined effects of copper, zinc and iron was more noxious to aquatic organisms than that of each metal applied singly.

The presence of 0.15 mgl^{-1} zinc and 0.15 mgl^{-1} lead had little effect on Potamophylax latipennis in the larval stage and Jones (1940) reports taking specimens of Stenophylax stellatus = Potamophylax latipennis from the River Ystwyth in water containing 0.9 mgl^{-1} zinc and 0.05 mgl^{-1} lead. Rehwold, Lasko, Shaw and Wirhowski (1973) list the 96 hour tolerance limit for " caddis fly(Trichoptera), unidentified" as 58.1 mgl^{-1} for zinc, whilst Clubb, Gaufin and Lords (1975) were unable to determine the 96 hour TL_m for the cased trichopteran Brachycentrus americanus exposed to cadmium and concluded that B. americanus is relatively insensitive to this metal; but Brown (1977) suggests that case dwelling trichopteran larvae are protected to some extent by the case from the effects of metallic pollution.

Earlier work by Hynes (1961), Macan (1957) and Elliott (1967) indicates that eggs of certain ephemeropterans may be present in a stream system for an extended period of time. Illies (1959) found that the eggs of Baetis took up to 297 days to hatch under laboratory conditions and Elliott(1967) suggests that this phenomenan of delayed hatching could cause serious errors in the calculation of growth rates. Elliott

(1967) also says that life histories differ from place to place and also from year to year and relates much of this variation to temperature.

The effects of small amounts, 0.15 mgl^{-1} of lead or zinc acting singly in the incubation medium, was to reduce the hatching time of eggs in the laboratory, and the combined effect of these metals was to inhibit hatching completely; eggs of all species incubated in water from the Glendhoo stream also failed to hatch, this is unlikely to be due to low oxygen tension (Hayes, Wilmot and Livingstone, 1951), as the exovation success was high in the control solution.

Evidence of the susceptibility of a particular stage in the life cycle of aquatic organisms is well documented in the literature (Grande, 1966; Brungs,1969). McKim and Benoit (1971)record that 17.5 mgl^{-1} copper had a toxic effect on trout fry but had no effect on the hatching success of eggs or on the growth rate of adults, and studies by Biesinger and Christensen (1972) showed that the sensitivity of some aquatic invertebrates to metals is similar to that of fish.

The observations recorded in this chapter suggest that hatching, moulting or pupation are the stages most susceptible to the effects of lead and zinc. Each of these stages is accompanied by changes in membrane integrity and considerable enzyme activity, and the presence of the metal ions may affect the enzymes; this is why it seems that moulting is such a vulnerable stage.Activity of enzymes or their co-factors may have been inhibited and hatching of eggs prevented; or the activity may have been enhanced, and this may have led to premature pupal emergence of the teneral adults of Potamophylax latipennis.

Variation of cuticle permiability with the moult has been demonstrated by previous workers (Anderson,1950; Lockwood and Inman,1973), and Yager and Harry (1964) associated the presence of heavy metal ions with membrane disruption.The results of the present investigation also imply changes in membrane permiability in the presence of the metals lead and zinc.This suggests that further work is needed in order to investigate the effects of different concentrations of heavy metal ions on the membrane permiability of aquatic invertebrates at different stages of their development.

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSIONS

The major point to have emerged from this study is the difference in fauna between two adjacent and very similar streams that are part of the same catchment in the Isle of Man, the Gleneedle stream and the Glendhoo stream. The two streams are very near to each other and physically similar, consequently it would be reasonable to expect their macrofauna to be similar also, but the Gleneedle stream has a varied fauna typical of Manx upland streams (Pugh-Thomas, 1974; Hynes, 1952, 1954) and the fauna of the Glendhoo stream is impoverished.

Over a two year period no lead or zinc was detected in the Gleneedle stream so these ions are assumed to be absent from its waters. However, the metals were always present in the Glendhoo stream ranging from 0.10 mgl^{-1} to 0.60 mgl^{-1} with an annual mean of 0.26 mgl^{-1} for lead; to an annual mean of 0.108 mgl^{-1} within the range 0.04 mgl^{-1} to 0.20 mgl^{-1} for zinc. The existence of these metals in the Glendhoo waters may be attributed to leachate from the spoil heaps of disused mines situated in the catchment area of the stream. The presence of heavy metal ions has been reported by previous workers, (Carpenter, 1924; Laurie and Jones, 1938; Jones, 1940 1941, 1950, 1958, 1964; Abdullah and Royle, 1972; Furmanska, 1979) to impose severe constraints on the ecology of fresh water stream and river systems. Carpenter (1924) observed a poverty of fauna in certain rivers in the Aberystwyth district of Wales, she related this to the proximity of lead mines and to the presence of lead in the stream waters in the order of 0.2 mgl^{-1} to 0.5 mgl^{-1}.

The River Ystwyth was also studied by Jones (1940) who observed that it still suffered from the pollutant effects of mining forty years after the cessation of these activities. He records that presence of both lead and zinc in the Ystwyth, in the order of 0.05 mgl^{-1} lead and 1.2 mgl^{-1} zinc, and reports that the fauna is of a limited nature restricted to insects, with numerous Plecoptera. Jones (1940) recorded 2 species of Ephemeroptera (*Baetis* spp and *Leptophlebia* spp), 6 species of Plecoptera (*Perla* spp, *Chloroperla* spp, *Perlodes*, *Leuctra* spp, *Isopteryx* and *Nemoura* spp) and 7 species of Trichoptera (*Rhyacophila dorsalis*, *Hydropsyche instabilis*, *Hydropsyche pellucidula*, *Polycentropus flavomaculatus*, *Philopotamus montanus*, *Sericostoma personatum* and *Stenophylax stellatus* = *Potamophylax latipennis* in water containing 0.05 mgl^{-1} lead and 0.9 mgl^{-1} zinc. Of these only *Potamophylax latipennis* = *Stenophylax stellatus* is common to both the Ystwyth and the Glendhoo waters; and only *Baetis* spp, *Nemoura* spp, *Rhyacophila dorsalis*, *Hydropsyche instabilis*, *Polycentropus flavomaculatus* and *Potamophylax latipennis* = *Stenophylax stellatus* are common to both the Ystwyth and Gleneedle waters. *Leptophlebia*, *Perla*, *Perlodes* and *Isopteryx* spp are not recorded in Manx waters.

Jones (1940) notes that he only collected one larva of *Stenophylax stellatus* = *Potamophylax latipennis* from the Ystwyth in a total of 5 sampling occasions compared with 16 larvae collected on the same number of occasions from the unpolluted River Dovey (Jones, 1941). Flood conditions are reported by Jones (1940) to reduce the zinc contents to 0.2 to 0.3 mgl^{-1} and render lead undetectable in the waters of the Ystwyth; this is in direct contrast to the behaviour of these metals in the Manx stream, where lead was always present in greater quantities than zinc, and flood conditions elevated the amount of both metals in the Glendhoo stream.

Jones (1958) conducted a further study on the River Ystwyth some 15 years later and concluded that it continued to be polluted by zinc in the order of 0.6 mgl^{-1} but lead pollution had become negligible. He lists 9 species of Plecoptera (Brachyptera risi, Amphinemura cinerea Protonemura meyeri, Leuctra inermis, L. hippopus, L. fusciventris, Chloroperla tripunctata, C. torrentium and Isoperla grammatica), 6 species of Ephemeroptera (Ephemerella ignita, Baetis rhodani, B. pumulis, Rhithrogena semicolorata, Heptagenia lateralis and Caenis sp), and 9 species of Trichoptera (Goera pilosa, Sericostoma personatum, Lepidostoma hirtum, Stenophylax stellatus = Potamophylax latipennis, Hydropsyche instablis, Plectrocnemia conspersa, Polycentropus flavomaculatus, Rhyacophila dorsalis and Glossosoma boltoni) in a qualitative collection made in a section of the river with an average current velocity of 2ft/sec (= 0.61 $m.s^{-1}$) and average daily flow of 28.8 cu. ft/sec (= 0.816 $m^3 s^{-1}$). Brooker and Morris (1980), record an average daily flow of 5.08 $m^3 s^{-1}$ for a stretch of the Ystwyth slightly upstream of Jones's (1958) station. Both these values are considerably greater than those of the Glendhoo stream which has an average daily flow of 0.012 $m^3 s^{-1}$ under normal conditions and 0.06$m^3 s^{-1}$ in flood (see appendix table A2).

Brooker and Morris (1980) extended the earlier work of Carpenter (1924) and Jones (1940, 1958) by considering the influence of lead and zinc on the riffle fauna of the rivers Ystwyth and Rheidol. They found 0.098 mgl^{-1} lead and 2.002 mgl^{-1} zinc in the most heavily polluted site on the Ystwyth and record that the fauna comprised 18.7% Plecoptera, 37.8% Ephemeroptera and 5.2% Trichoptera at this station. At a similar site on the Rheidol, with 0.010 mgl^{-1} lead and 0.336 mgl^{-1} zinc the faunal density comprised 49.9% Plecoptera 31.8% Ephemeroptera and 4.0% Trichoptera. The average daily flow at these sites was recorded as 0.35 m^3s^{-1} and 0.34 m^3s^{-1} respectively which are clearly greater than that of the Manx stream. The lowest metal concentrations recorded by Brooker and Morris are 0.005 mgl^{-1} lead in association with 0.015 mgl^{-1} zinc in the Ystwyth and 0.004 mgl^{-1} lead found with 0.012 mgl^{-1} zinc in the Rheidol, the proportional representation of taxonomic groups are given as Plecoptera 28.6% and 49.9% Ephemeroptera 15.5% and 31.8% and Trichoptera 4.1% and 4.0% respectively for the Ystwyth and Rheidol. Brooker and Morris do not list the species collected at each station and it would have been interesting to compare their findings with those of Carpenter (1924), Jones (1940, 1958) and with the Manx stream.

The main difference apart from taxa and flow rate, between the Welsh rivers Ystwyth and Rheidol, and the Manx Glendhoo stream appears to lie in the ratio of lead to zinc; as shown in table 7.1, the ratio varies from a minimum of 0.05 to a maximum of 0.33 with a mean 0.13 and variance of 0.1 in the Ystwyth. In the Rheidol the ratio of lead to zinc varies from a minimum of 0.03 to a maximum of 0.33 with a mean of 0.09 and variance of 0.1 (values calculated from data recorded by Brooker and Morris,1980, and fully tabulated in the appendix table A18). The ratio of lead to zinc in the Glendhoo stream has a mean value of 2.5 and a variance of 0.5 within the range 1.5 to 4.5 (see appendix table A3) this is clearly a much higher ratio in the Manx waters than it is in the Welsh rivers - see table 7.1 and appendix table A18. A high ratio of lead to zinc is also evident in certain Cornish rivers,

RIVER	SOURCE OF REFERENCE	METAL CONCENTRATION $mg.l^{-1}$ LEAD	ZINC	RATIO LEAD/ZINC
RHEIDOL	BROOKER AND MORRIS (1980)	0.004 0.009	0.012 0.327	0.33 0.03
YSTWYTH	BROOKER AND MORRIS (1980)	0.005 0.098	0.015 2.002	0.33 0.05
YSTWYTH	JONES (1958)	<0.10 -	0.60 0.50	0.16 -
YSTWYTH	JONES (1940)	0.05 0.02	0.90 1.20	0.05 0.02
GANNEL	ASTON AND THORNTON (1977)	0.052 0.011	0.048 0.458	1.08 0.02
FOWEY	ASTON AND THORNTON (1977)	0.003 0.001	0.001 0.007	3.00 0.14
GLENDHOO STREAM	-	0.18 0.15	0.04 0.10	4.50 1.50

TABLE 7.1. Maximum and minimum values of the ratio of lead to zinc in the Welsh rivers Rheidol and Ystwyth, the Cornish rivers Gannel and Fowey, and the Manx Glendhoo stream.

see table 7.1. The ratio of lead to zinc varies from a minimum of 0.02 to a maximum of 1.08 with a mean of 0.5 and variance of 0.13 in the River Gannel which has its catchment in a mining area; whilst the River Fowey, which drains an unmineralised area, has a mean lead to zinc ratio of 0.8 and a variance of 0.7 within the range of 0.14 to 3.00 (values calculated from data recorded by Aston and Thornton, 1977). It should be noted however, that the concentrations of lead and zinc are very much lower in the Fowey than those in the Gannel.

Norris, Lake and Swain (1980) suggest that ground water inflow may control the input of some metal ions into a river and Mackay and Schnellman (1963) record that the mineral veins of Cross's mine were very narrow, "often not commercially viable to work, but exceptionally rich in lead ore, almost solid galena with very little gangue material." This may account for the high concentrations of lead relative to zinc in the Glendhoo stream.

The calcium content of the Glendhoo stream varied between 2.0 to 6.5 mgl^{-1} compared with 3.0 to 4.4 mgl^{-1} for the Ystwyth (Jones, 1940). Both Klein (1957) and Jones (1964) have demonstrated that lead and zinc are more toxic to aquatic life in soft waters and as the calcium content of the Ystwyth and Glendhoo are similar, it is not unreasonable to expect that similar concentrations of the same metal would be equally toxic in both waters, but this does not seem to be the case.

Carpenter (1924) has testified to the harmful effects of lead to aquatic life, whilst Laurie and Jones (1938) associated a reduction in the amounts of lead with improvements in the invertebrate fauna in the Rheidol, and Jones (1940) records that zinc pollution may be as destructive as lead to fish, but certain insect species appear to be tolerant of pollution by zinc; however Brooker and Morris (1980) state that there was no evidence that the distribution of fauna in the Rheidol and Ystwyth was related simply to contamination by zinc, but Furmanska (1979) demonstrated that the synergistic effects of two or more metals on aquatic invertebrates was much greater than the contribution of each metal on its own. Brown (1976) in a series of experiments with the isopod *Asellus meridianus* has shown that tolerance to copper may also confer tolerance to lead, but adaptation to lead, in *Asellus*, did not appear to be accompanied by a tolerance to copper. Brown suggests that different mechanisms of tolerance operate for each metal and that metal tolerance may be a genetic factor.

This suggests that the relative proportions of lead and zinc, in the mean ratio of 2.5 in the Glendhoo stream, may be more harmful than they are in the Welsh rivers where the ratio is in the order of 0.02 to 0.33 and that lead is the more critical ion in the Manx stream.

It is a reasonable conclusion that the presence of small but varying amounts, of lead and zinc throughout the year in the Glendhoo stream exert a controlling influence on the ecology of this stream by acting synergistically to inhibit the exovation of aquatic insect eggs, thus preventing colonization of the water by insects from nearby streams.

The presence of apparently healthy larvae of *Potamophylax latipennis* in the Glendhoo stream is of particular interest, as it is in direct contrast to laboratory observations where eggs of *P. latipennis* failed to develop in water taken from the Glendhoo stream, and also failed to hatch under the combined influence of lead and zinc. This contradictory situation may be connected with the ovipositing behaviour of the female imago.

Adults of _P. latipennis_ were particularly active on damp evenings, especially during periods of light or impending rain, and gravid females were observed to oviposit on vegetation which overhung the Glendhoo stream. Crichton (1961) suggests that rainfall acts as a stimulus to egg laying behaviour and that there may be a connection between rainfall and egg laying sites out of the water for Limnephilidae. Moseley (1939) records similar behaviour by caddis and says "in some cases eggs are placed on overhanging leaves or twigs, the young larvae dropping off directly into the water" and Hickin (1967) records Wesenberg-Lund "observing sticky drops, containing Limnephilid larvae falling from a tree during a rainstorm with recently emerged larvae swimming in the water below." Clearly, this choice of egg laying site, with its dependence upon associated rainfall, would permit _P. latipennis_ larvae to emerge in a medium free from contamination by heavy metal ions. Laboratory observations confirmed that mature _P. latipennis_ larvae exhibit a tolerance towards the presence of lead and zinc. Laboratory results also suggest that the metal ions present in the Glendhoo stream act on _P. latipennis_ during pupal metamorphosis to inhibit normal wing development, thus preventing flight by the adult insect. Observations also suggest that the synergistic effects of lead and zinc influence _P. latipennis_ to reduce the time spent in pupation, resulting in an earlier but less successful emergence of teneral adults. These observations may explain why the maximum population density of _P. latipennis_ occurred one month earlier in the Glendhoo stream than in the Gleneedle stream during 1978 and 1979; and may also explain the numbers of dead pupae of *P. latipennis* found in the Glendhoo stream during the same period. Nielsen (1974) also records finding small numbers of *P. latipennis* in the polluted Danish River Lindenborg but states that all pupae found were dead. Nielsen suggests that the presence of the larvae was due to immigration of adults from nearby but less polluted streams.

Similar factors may be operating in both the River Lindenborg and in the streams of the Gleneedle area of the Isle of Man.

The problem of seepage from old mine workings is likely to remain and affect the Glendhoo stream for some considerable time, especially during periods of high surface run off. Laurie and Jones (1938) consider that extended periods of heavy rain in mining areas might maintain the metal content of a river at a sufficiently high concentration to destroy some of the fauna, but Clubb, Gaufin and Lords (1975) are of the opinion that mature aquatic insect larvae may recover from a metal pulse in a river.

Work by Carpenter (1924), Laurie and Jones (1938), Jones (1940, 1958) and Brooker and Morris (1980) on the effects of metal contamination on the biota of rivers in Welsh mining areas indicate that there has been a gradual but significant reduction in the presence of metal ions in these rivers resulting in a considerable improvement in water quality and biological status over the past fifty or so years.

Similar attenuating mechanisms should be operating in the Isle of Man and it may prove an interesting study to measure the concentrations of lead and zinc and sample the fauna of the Glendhoo stream at intervals in the future to compare them with the findings of this study.

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APPENDIX

CONTENTS OF APPENDIX

METHODS USED FOR COLLECTION AND ANALYSIS OF WATER AND SEDIMENT SAMPLES

Methods of Collection

Sediment Samples

Sediment samples were obtained from the edges of pools using a 5 cm diameter plastic corer, filtered through a nylon mesh sieve of 600 μm aperture and placed into polythene containers.

Water Samples

Two 500 ml acid washed (i.e. soaked for 48 hours in a 10% solution of nitric acid) polythene bottles were flushed four times with stream water, then filled to the brim with stream water filtered through a nylon mesh of 300 μm aperture; one bottle was acidified with 2 ml of concentrated nitric acid to prevent adsorbtion of metal ions onto the container walls. The acidified samples was used to determine the concentration of metal ions in solution the other sample used to determine dissolved oxygen content, biochemical oxygen demand, nitrate nitrogen concentration and pH.

Methods of Analysis

Metal ions (Ca, Mg, Pb, Zn, Cu, and Fe)

The sediments were dried to a constant weight at 80°C and passed through nylon sieves of 400 μm and 200 μm, the 200 μm being retained.

0.5g of sediment was digested in 15 ml concentrated nitric acid at 20°C and left for approximately 8 - 10 hours. The solution was then made up to 50 ml with double distilled water and analysed for lead, copper and zinc by atomisation using a Pye Unicam SP90A series 2 atomic absorption spectrophotometer with limits of detection 0.001 μgl^{-1} Zn and 0.02 μgl^{-1} lead, appropriate cathode lamps and standard blank solutions.

The concentration of lead, zinc, iron or sodium in the stream water was measured by placing the 'sipper tube' of the atomic absorption photospectrometer directly into the appropriate sample bottle. Because of interference effects 1 ml of 5% lanthium solution (Golterman, Clymo and Ohnstad, 1978) was added to every 4 ml of stream water before measuring the concentration of magnesium or calcium present using the atomic absorption photospectrometer.

Biochemical Oxygen Demand

The procedures used were those described by Golterman, Clymo and Ohnstad (1978) and are only briefly described here.

250ml of the sample water was diluted by the addition of 250 ml of distilled water, the pH was adjusted, if necessary, to 6.5; nutrients and nitrification inhibitor were added. Two x 250 ml bottles were filled with this solution and incubated in the dark for 5 days at 20°C. The oxygen concentration was measured before and after incubation using an Electronic Industries Limited (E.I.L.) oxygen probe.

Nitrate Nitrogen

25ml of the stream water sample was prepared according to the method described in detail, by Mackereth, Heron and Talling (1978). The absorbance of the sample was then measured using a Unicam SP 500 spectrophotometer, a 1 cm cell and a wavelength of 543 nm. The nitrate nitrogen concentration was then obtained by reference to the appropriate calibration graph.

	1978											1979														
STREAM	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	MEAN	VARIANCE
GLENEEDLE	20	30	47	38	27	18	3	2	2	-	4	6	15	14	28	27	21	16	7	5	6	3	4	7	15.22	157.74
GLENDHOO	18	28	4	1	2	4	5	8	10	-	5	11	16	20	4	6	3	4	5	6	2	6	7	13	8.17	44.138

TABLE A1. Numbers of Potamophylax latipennis sampled during 1978 and 1979. Figures shown are the totals obtained by adding the numbers collected at each of the three collecting stations.

																								Totals
GLENEEDLE	3½	3½	7	7	11½	11½	16½	21	21	25	25	31	32	33½	35½	37½	39	40½	40½	42½	44	45	46	R1 = 619.5
GLENDHOO	1	3½	3½	7	11½	11½	11½	11½	16½	16½	16½	21	21	21	25	27	28	29	30	33½	35½	37½	42½	R2 = 461.5

TABLE A1a. Ranked data from TABLE A1.

Dispersion of Potamophylax latipennis in the Glendhoo and Gleneedle Gleneedle streams.

Variance to mean ratio (Elliott, 1977).

Gleneedle stream $\quad {}^{2} = \frac{S^2 \ (n - 1)}{\bar{x}} = 228.0$

Glendhoo stream $\quad {}^{2} = \frac{S^2 \ (n - 1)}{\bar{x}} = 118.9$

Using data from table A1 and Fig. 8 of Elliott (1977), degrees of freedom, $V = n - 1 = 22$ and for each case χ^2 is well above the upper 5% significance limit indicating that the dispersion of P. latipennis is contagious in each stream.

Using the ranked data from table A1(a) and applying the non parametric Mann-Whitney U test with null hypothesis H_o,

H_o = the distribution and the median values of P. latipennis are the same for each stream.

$$U_1 = n_1 \ n_2 + \tfrac{1}{2} \ n_2 \ (n_2 + 1) \ - \ R_2 \ = \ 343.5$$

$$U_2 = n_1 \ n_2 + \tfrac{1}{2} \ n_1 \ (n_1 + 1) \ - \ R_1 \ = \ 185.5$$

As $n_1 = n_2 > 20$ the 'U' statistic is transformed to a normal variate 'd'

$$d = \frac{U - (n_1 \ n_2 \ / \ 2)}{\sqrt{\dfrac{n_1 \ n_2 \ (n_1 + n_2 + 1)}{12}}} = 1.736$$

from tables of normal distribution $d > 1.65$ therefore H_o is rejected at the 90% level and the difference between the populations of P. latipennis in the Gleneedle and Glendhoo streams is significant ($P < 0.1$).

STREAM	PARAMETER		1978										1979												
			F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J
GLENEEDLE	SURFACE	STATION NO. 1	.08	-	-	-	.05	-	-	.03	-	-	-	-	-	.04	-	-	-	-	.18	-	-	.23	-	-
	CURRENT	STATION NO. 2	.07	-	-	-	.04	-	-	.02	-	-	-	-	-	.04	-	-	-	-	.18	-	-	.22	-	-
	VELOCITY	STATION NO. 3	.07	-	-	-	.04	-	-	.02	-	-	-	-	-	.04	-	-	-	-	.15	-	-	.19	-	-
	$m.s.^{-1}$	MEAN VALUE	.07	-	-	-	.04	-	-	.02	-	-	-	-	-	.04	-	-	-	-	.17	-	-	.21	-	-
	AVERAGE DAILY FLOW m^3s^{-1}		.021	-	-	-	.012	-	-	.006	-	-	-	-	-	.012	-	-	-	-	.051	-	-	.063	-	-
GLENDHOO	SURFACE	STATION NO. 1	.07	.10	.09	.10	.04	.19	.02	.02	.11	.26	.17	.12	.06	.03	.05	.04	.07	.25	.16	.03	.03	.21	.13	.15
	CURRENT	STATION NO. 2	.07	.12	.11	.11	.07	.19	.03	.02	.13	.30	.18	.13	.08	.04	.06	.06	.07	.27	.18	.04	.03	.23	.15	.18
	VELOCITY	STATION NO. 3	.05	.09	.09	.09	.03	.20	.02	.03	.10	.21	.15	.09	.05	.04	.05	.03	.05	.22	.15	.03	.03	.21	.12	.15
	$m.s.^{-1}$	MEAN VALUE	.07	.10	.09	.10	.05	.19	.02	.02	.11	.26	.17	.11	.06	.04	.05	.04	.06	.25	.16	.03	.03	.22	.13	.16
	AVERAGE DAILY FLOW m^3s^{-1}		.021	-	-	-	.015	-	-	.006	-	-	-	-	-	.009	-	-	-	-	.048	-	-	.066	-	-

TABLE A2. Seasonal variations in surface current velocity and average daily flow from January 1978 to February 1980. The variance to mean ratio (Elliot 1977) is used to test the surface current velocity of the Glendhoo stream for agreement with a contagious distribution. $\chi^2 = 204.7$, as 'n' is large, χ^2 is transformed to a normal variate 'd'. $d = \sqrt{2\chi^2} - \sqrt{2\nu - 1} = 8.36$ where ν = n-1 degrees of freedom: The statistic 'd' = 8.36 is greater than 2.58 indicating that the distribution is contagious at the 99% level.

	CONCENTRATION mg. l^{-1}																								MEAN	VAR.
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J		
LEAD	.15	.30	.15	.30	.15	.50	.10	.10	.18	.60	.40	.35	.15	.10	.15	.10	.12	.70	.11	.15	.15	.60	.30	.40	.263	.032
ZINC	.05	.16	.06	.20	.05	.20	.05	.05	.04	.20	.18	.15	.05	.06	.10	.03	.05	.20	.05	.08	.05	.18	.20	.15	.108	.004
RATIO LEAD/ZINC	3.0	1.9	2.5	1.5	3.0	2.5	2.0	2.0	4.5	3.0	2.2	2.3	3.0	1.7	1.5	3.3	2.4	3.5	2.2	1.9	3.0	3.3	1.5	2.7	2.5	0.5

TABLE A3. Seasonal variations in the concentrations of lead and zinc in the Glendhoo stream from February 1978 to January 1980.

LEAD x	.10	.10	.10	.10	.12	.12	.15	.15	.15	.15	.15	.15	.15	.18	.30	.30	.30	.35	.40	.40	.50	.60	.60	.70
ZINC y	.05	.05	.06	.03	.05	.05	.08	.05	.10	.05	.05	.06	.10	.04	.16	.20	.20	.15	.15	.18	.20	.18	.20	.20
P	15	15	13	23	15	15	12	15	10	15	15	13	10	22	7	0	0	8	8	5	0	5	0	0
Q	2	2	3	0	2	2	4	2	5	2	2	3	5	1	7	9	9	6	6	8	9	8	9	9
P-Q	13	13	10	23	13	13	8	13	5	13	13	10	5	21	0	-9	-9	2	2	-3	-9	-3	-9	-9

TABLE A3(a) Ranked data for concentration of lead taken from TABLE A3.

Degree of Association Between Concentration of Lead and Concentration of Zinc in the Glendhoo stream from February 1978 to January 1980.

Applying Kendall's coefficient of rank correlation to the ranked data in table A3(a).

$S = \sum P-Q = 126$

$n = 24$

$\varepsilon_1 = 7, \varepsilon_2 = 2, \varepsilon_3 = 3, \varepsilon_4 = 5, \varepsilon_5 = 2, \varepsilon_6 = 2$

$$X = \tfrac{1}{2}[\varepsilon_1(\varepsilon_1 - 1) + \varepsilon_2(\varepsilon_2 - 1) + \varepsilon_3(\varepsilon_3 - 1) + \varepsilon_4(\varepsilon_4 - 1) + \varepsilon_5(\varepsilon_5 - 1) + \varepsilon_6(\varepsilon_6 - 1)] = 37$$

$\eta_1 = 7, \eta_2 = 2, \eta_3 = 2, \eta_4 = 2, \eta_5 = 2, \eta_6 = 5$

$$Y = \tfrac{1}{2}[\eta_1(\eta_1 - 1) + \eta_2(\eta_2 - 1) + \eta_3(\eta_3 - 1) + \eta_4(\eta_4 - 1) + \eta_5(\eta_5 - 1) + \eta_6(\eta_6 - 1)] = 35$$

$$\tau = S / \sqrt{\tfrac{1}{2} n (n-1) - X} \times \sqrt{\tfrac{1}{2} n (n-1) - Y} = 0.53$$

The value of τ indicates a positive association between the concentration of lead and the concentration of zinc in the Glendhoo stream.

MEAN WATER VELOCITY $m.s^{-1}$ x	.02	.02	.03	.03	.03	.04	.05	.05	.06	.06	.07	.09	.10	.10	.11	.11	.13	.16	.16	.17	.19	.22	.25	.26
LEAD $mg.l^{-1}$ y	.01	.10	.10	.15	.15	.10	.12	.15	.12	.15	.15	.15	.30	.30	.18	.35	.30	.15	.40	.40	.50	.60	.70	.60
P	20	20	20	11	11	13	16	11	16	11	11	11	7	7	10	6	7	11	4	4	3	1	0	
Q	0	0	0	2	2	0	1	2	1	2	2	2	4	4	3	5	4	2	6	6	7	8	9	
P-Q	20	20	20	9	9	20	15	9	15	9	9	9	3	3	7	1	3	9	-2	-2	-4	-7	-9	

TABLE A3(c) showing ranked data for water velocity (from TABLE A2) and Lead concentration (TABLE A3).

Applying Kendall's coefficient of rank, correlation to the ranked data

$S = \sum(P-Q) = 166$, $n = 24$

$\varepsilon_1 = 2, \varepsilon_2 = 3, \varepsilon_3 = 2, \varepsilon_4 = 2, \varepsilon_5 = 2, \varepsilon_6 = 2, \varepsilon_7 = 2$

$X = \frac{1}{2} [\varepsilon_1 (\varepsilon_1-1) + \varepsilon_2 (\varepsilon_2-1) + \varepsilon_3 (\varepsilon_3-1) + \varepsilon_4 (\varepsilon_4-1) + \varepsilon_5 (\varepsilon_5-1) + \varepsilon_6 (\varepsilon_6-1) + \varepsilon_7 (\varepsilon_7-1) = 9$

$\eta_1 = 5, \eta_2 = 2, \eta_3 = 7, \eta_4 = 3, \eta_5 = 2, \eta_6 = 2$

$Y = \frac{1}{2} [\eta_1 (\eta_1-1) + \eta_2 (\eta_2-1) + \eta_3 (\eta_3-1) + \eta_4 (\eta_4-1) + \eta_5 (\eta_5-1) + \eta_6 (\eta_6-1) = 37$

$\tau = S / \sqrt{(\frac{1}{2} n (n-1) - X)} \times \sqrt{(\frac{1}{2} n (n-1) - Y)} = 0.66$

Clearly the coefficient τ indicates a good degree of association between mean water velocity and concentration of lead in the Glendhoo stream.

STREAM	STATION NO.	pH																							
		F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J
GLENEEDLE	1	6.0	6.1	6.0	6.4	6.3	6.2	6.0	6.1	6.0	6.0	5.9	5.9	6.0	6.0	6.1	6.0	6.3	6.2	6.4	6.2	6.2	6.0	5.9	5.9
	2	6.0	6.2	6.1	6.4	6.2	6.2	6.0	6.0	6.1	5.9	5.9	5.9	6.0	6.0	6.1	5.9	6.2	6.2	6.4	6.2	6.2	6.0	5.8	5.9
	3	5.9	6.1	6.1	6.4	6.3	6.2	6.0	6.1	6.1	5.9	5.9	5.9	6.0	6.0	6.0	5.9	6.3	6.2	6.3	6.2	6.2	6.0	5.9	5.9
GLENDHOO	4	5.9	6.1	6.1	6.4	6.4	6.2	6.0	6.1	6.2	5.9	5.9	5.9	6.0	6.0	6.1	5.9	6.2	6.2	6.2	6.2	6.2	6.0	5.9	5.9
	5	6.0	6.0	6.2	6.3	6.3	6.2	6.0	6.1	6.1	5.9	5.9	5.9	5.9	6.0	6.1	5.9	6.2	6.2	6.4	6.2	6.2	6.0	6.0	5.9
	6	6.0	6.1	6.1	6.4	6.3	6.2	6.0	6.0	6.1	5.9	5.9	5.9	6.0	6.0	6.1	5.9	6.2	6.2	6.4	6.2	6.2	6.0	5.9	5.9

TABLE A4. Seasonal variations in pH in the Gleneedle and Glendhoo streams from February 1978 to January 1980 and expressed in the usual units.

MEAN WATER VELOCITY ms^{-1}	x	.02	.02	.03	.03	.03	.04	.05	.05	.06	.06	.07	.09	.10	.10	.11	.11	.13	.16	.16	.17	.19	.22	.25	.26
pH	y	6.0	6.0	6.1	6.2	6.2	5.9	6.3	6.1	6.2	6.0	6.0	6.1	6.1	6.4	6.1	5.9	5.9	6.4	5.9	5.9	6.2	6.0	6.2	5.9
	P	14	14	8	3	3	18	2	8	3	14	14	8	8	0	8	18	18	0	18	18	3	14	3	18
	Q	1	1	2	3	3	0	4	2	3	1	1	2	2	5	2	0	0	5	0	0	3	1	3	0
	P-Q	13	13	6	0	0	18	-2	6	0	13	13	6	6	-5	6	18	18	-5	18	18	0	13	0	18

TABLE A4(a) Mean water velocity (from TABLE A2) ranked with pH (from TABLE A4).

Degree of Association Between Surface Current Velocity and pH in the Glendhoo stream.

Applying Kendall's coefficient of rank correlation τ to the ranked data in table A4(a).

$$\varepsilon_1 = 2, \varepsilon_2 = 3, \varepsilon_3 = 2, \varepsilon_4 = 2, \varepsilon_5 = 2, \varepsilon_6 = 2, \varepsilon_7 = 2$$

$$Y = \tfrac{1}{2} [\varepsilon_1 (\varepsilon_1 -1) + \varepsilon_2 (\varepsilon_2 -1) + \varepsilon_3 (\varepsilon_3 -1) + \varepsilon_4 (\varepsilon_4 -1) + \varepsilon_5 (\varepsilon_5 -1)$$

$$+ \varepsilon_6 (\varepsilon_6 -1) + \varepsilon_7 (\varepsilon_7 -1) = 9$$

$$\eta_1 = 6, \eta_2 = 4, \eta_3 = 6, \eta_4 = 5, \eta_5 = 2$$

$$Y = \tfrac{1}{2} [\eta_1(\eta_1 -1) + \eta_2 (\eta_2 -1) + \eta_3 (\eta_3 -1) + \eta_4 (\eta_4 -1) + \eta_5 (\eta_5 -1)$$

$$= 47$$

$$S = \sum P-Q = 191 \qquad n = 24$$

$$\tau = S / \sqrt{(\tfrac{1}{2} n (n -1) - X)} \quad x \quad / \overline{(\tfrac{1}{2} n (n -1) - Y} = 0.77$$

Clearly this value of $\tau = 0.77$ indicates a good degree of correlation between surface current velocity and pH in the Glendhoo stream.

Correlation between B.O.D., Nitrate nitrogen, Magnesium, Calcium and Sodium content of the Gleneedle and Glendhoo streams.

Applying the Mann-Whitney 'U' test, and ranking the data from table 5.1.

$$U_1 = n_1 n_2 + \frac{n_2 (n_2 (n_2 + 1)}{2} - R_2 \qquad n_1 = n_2 = 6$$

$$U_2 = n_1 n_2 + \frac{n_2 (n_2 + 1)}{2} - R_1$$

Ranked data for Biochemical Oxygen Demand.

Gleneedle	$1\frac{1}{2}$	$3\frac{1}{2}$	$5\frac{1}{2}$	8	10	12	$R_1 = 40\frac{1}{2}$
Glendhoo	$1\frac{1}{2}$	$3\frac{1}{2}$	$5\frac{1}{2}$	8	8	11	$R_2 = 37\frac{1}{2}$

$U_1 = 19.5, \quad U_2 = 16.5$

Ranked data for Nitrate Nitrogen Concentration

Gleneedle	$1\frac{1}{2}$	$3\frac{1}{2}$	$6\frac{1}{2}$	$6\frac{1}{2}$	11	12	$R_1 = 41$
Glendhoo	$1\frac{1}{2}$	$3\frac{1}{2}$	$6\frac{1}{2}$	$6\frac{1}{2}$	9	10	$R_2 = 37$

$U_1 = 20, \quad U_2 = 16$

Ranked data for Magnesium Concentration

Gleneedle	$2\frac{1}{2}$	5	$6\frac{1}{2}$	8	10	10	$R_1 = 42$
Glendhoo	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$6\frac{1}{2}$	10	12	$R_2 = 36$

$U_1 = 21, \quad U_2 = 15$

Ranked data for Calcium Concentration

Gleneedle	$1\frac{1}{2}$	4	5	$7\frac{1}{2}$	9	11	$R_1 = 38$
Glendhoo	$1\frac{1}{2}$	4	4	$7\frac{1}{2}$	11	11	$R_2 = 39$

$U_1 = 18, \quad U_2 = 19$

Ranked data for Sodium Concentration

Gleneedle	$2\frac{1}{2}$	$2\frac{1}{2}$	$5\frac{1}{2}$	$7\frac{1}{2}$	$9\frac{1}{2}$	$11\frac{1}{2}$	$R_1 = 39$
Glendhoo	$2\frac{1}{2}$	$2\frac{1}{2}$	$5\frac{1}{2}$	$7\frac{1}{2}$	$9\frac{1}{2}$	$11\frac{1}{2}$	$R_2 = 39$

$$U_1 = U_2 = 18$$

Null Hypothesis H_o = There is no difference in the Median Values B.O.D., Nitrate nitrogen, Magnesium, Calcium and Sodium Concentration between samples from the Gleneedle stream, and samples from the Glendhoo stream, and the samples are drawn from the same population.

The tabulated values of the statistic 'U' (Elliott,1977) are greater than the calculated value of U_1 or U_2 in each case, therefore, H_o is accepted at the 95% level of significance ($P = 0.05$) and there is no difference in the B.O.D., Nitrate nitrogen, Magnesium, Calcium or Sodium concentrations in the two streams.

STREAM	STATION NO.	WATER TEMPERATURE °C																							
		F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J
GLENEEDLE	1	4.0	6.5	7.0	10	15	13	16	10	11	9.0	6.5	7.0	3.0	4.5	6.0	8.0	12	10	16	13	11	10	3.5	3.5
	2	4.0	6.5	7.0	10	15	13	16	10	11	9.0	6.5	7.0	3.0	4.5	6.0	8.0	12	10	16	13	11	10	3.5	3.5
	3	4.0	6.5	7.0	10	15	13	16	10	11	9.0	6.5	7.0	3.0	4.5	6.0	8.0	12	10	16	13	11	10	3.5	3.5
GLENDHOO	4	4.5	7.0	7.0	10	15	13	16	10	12	9.5	7.0	7.0	4.0	5.0	6.0	8.0	12	10	16	13	11	10	4.0	4.0
	5	4.5	7.0	7.0	10	15	13	16	10	12	9.5	7.0	7.0	4.0	5.0	6.0	8.0	12	10	16	13	11	10	4.0	4.0
	6	4.5	7.0	7.0	10	15	13	16	10	12	9.5	7.0	7.0	4.0	5.0	6.0	8.0	12	10	16	13	11	10	4.0	4.0

TABLE A5. Seasonal variations in water temperature in the Gleneedle and Glendhoo streams from February 1978 to January 1980.

STREAM	AIR TEMPERATURE °C																							
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J
GLENEEDLE	3	8.5	8.5	15	28	20	26	13	15	12	8	7	3	6	12	18	27	20	27	21	14	10	8	2
GLENDHOO	3	8.5	8.5	15	28	20	26	13	15	12	8	7	3	6	12	18	27	20	27	21	14	10	8	2

TABLE A6. Seasonal variations in air temperature above the Gleneedle and Glendhoo streams from February 1978 to January 1980.

STREAM	STATION NO.	DISSOLVED OXYGEN % SATURATION																							
		F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J
GLENEEDLE	1	95	90	90	85	81	81	81	85	80	87	90	85	89	90	86	82	82	81	81	85	87	95	92	90
	2	95	90	90	85	81	81	81	85	80	87	90	85	89	90	86	82	82	81	81	85	87	95	92	90
	3	95	90	90	85	80	80	80	85	80	85	90	85	90	90	85	82	82	80	80	85	87	95	92	90
GLENDHOO	4	95	90	90	85	80	81	80	85	80	87	90	85	89	90	85	82	82	80	80	85	87	95	92	90
	5	95	90	90	85	81	81	81	85	80	87	90	85	89	90	86	82	82	81	81	85	86	95	92	90
	6	95	90	90	85	81	81	81	85	89	87	90	85	89	90	86	82	82	80	80	85	85	95	90	90

TABLE A7 Seasonal variations in the dissolved oxygen concentration of the Gleneedle and Glendhoo streams from February 1978 to January 1980.

ZINC Concentration mgl^{-1}			LEAD Concentration mgl^{-1}			Percentage of Larvae Pupating	Number of days before Pupating		Percentage emerging from Pupation	Number of days during Pupation		Condition of Teneral Adult on Emergence
Init.	Final	Mean	Init.	Final	Mean		Range	Mean		Range	Mean	
0.15	0.13	0.14	0	0	0	100(20)	150-167	159	90(18)	37-43	40	3 Teneral adults had underdeveloped wings. 15 teneral adults apparently normal.
0	0	0	0.15	0.15	0.15	100(20)	151-169	160	95(19)	39-45	42	4 Teneral adults had underdeveloped wings. 15 Teneral adults apparently normal.
0.15	0.13	0.14	0.15	0.14	0.15	100(20)	138-152	146	80(16)	36-42	38	All Teneral adults had underdeveloped wings.
0.30	0.28	0.29	0	0	0	95(19)	78-92	86	85(17)	28-34	31	All Teneral adults had underdeveloped wings.
0	0	0	0.30	0.30	0.30	95(19)	81-97	90	90(18)	32-37	34	All Teneral adults had underdeveloped wings.
0.30	0.29	0.30	0.30	0.28	0.29	75(15)	52-63	57	70(14)	19-22	20	All Teneral adults had grossly distorted wings.
0.50	0.48	0.49	0	0	0	75(15)	27-32	30	65(13)	20-23	20	All Teneral adults had grossly distorted wings.
0	0	0	0.50	0.49	0.50	80(16)	26-32	30	60(12)	19-22	20	All Teneral adults had grossly distorted wings.
0.50	0.49	0.50	0.50	0.48	0.49	60(12)	21-27	25	0	-	-	Teneral adults had not emerged 50 days after entering pupation.
GLENDHOO STREAM WATER ONLY						100(20)	141-155	148	90(18)	33-38	36	Wings underdeveloped and did not open fully.
CONTROL: GLENEEDLE STREAM WATER ONLY						100(20)	158-181	169	100(20)	38-48	42	No apparent abnormalities.

TABLE A8. Observations of well grown Potamophylax latipennis larvae with a case length of 17mm - 22mm placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory. Each test comprised five samples of four larvae. Figures in brackets refer to the actual numbers.

Calculations of significance and association between experimental results for larvae of Potamophylax latipennis exposed to different concentrations of lead and zinc.

a) Number of larvae pupating

	METAL CONCENTRATION					Totals
	$0.5\ mg.l^{-1}$ Zinc	$0.5\ mg.l^{-1}$ Lead	$0.5\ mg.l^{-1}$ Zinc plus $0.5\ mg.l^{-1}$ Lead	Glendhoo Stream Water Only	Gleneedle Stream Water Only	
Observed Frequency f_o	15	16	12	20	20	83
Expected Frequency f_e	16.6	16.6	16.6	16.6	16.6	83

Data taken from fig.6.2

Null Hypothesis, H_o = there is no difference between the numbers of larvae pupating in each experimental solution.

$$\chi^2 = \sum \frac{(f_o - f_e)^2}{f_e} = 2.23 \text{ with 4 degrees of freedom}$$

The calculated value of χ^2 is less than the tabulated value of 7.8 (Fisher and Yates, 1963) so the null hypothesis is not rejected at the 10% level.

(b) Number of larvae of P. latipennis emerging from pupation

	METAL CONCENTRATION											
	0.15 $mg.l^{-1}$ Zinc	0.30 $mg.l^{-1}$ Zinc	0.50 $mg.l^{-1}$ Zinc	0.15 $mg.l^{-1}$ Lead	0.30 $mg.l^{-1}$ Lead	0.50 $mg.l^{-1}$ Lead	0.15 mgl Zinc + 0.15 mgl^{-1} Lead	0.30 mgl^{-1} Zinc + 0.30 mgl^{-1} Lead	0.50 mgl^{-1} Zinc + 0.50 mgl^{-1} Lead	Gleneedle Stream Water Only	Glendhoo Stream Water Only	Totals
OBSERVED FREQUENCY f_o	18	17	13	19	18	12	16	14	0	20	18	165
EXPECTED FREQUENCY f_e	15	15	15	15	15	15	15	15	15	15	15	165

Data taken from fig. 6.2

Null Hypothesis, H_o = there is no difference between the number of larvae emerging from pupation in each experimental solution.

$$\chi^2 = \sum \frac{(f_o - f_e)^2}{f_e} = 20 \text{ with 10 degrees of freedom}$$

The calculated value of ^{2} is greater than the tabulated value of 18.3 (Fisher and Yates, 1963) therefore H_o is rejected at the 5% level, and the differences between the number of larvae emerging in each solution is significant ($P<0.05$).

(c) Number of days spent in pupation by larvae of P. latipennis

	METAL CONCENTRATION											
	0.15 $mg.l^{-1}$ Zinc	0.30 $mg.l^{-1}$ Zinc	0.50 $mg.l^{-1}$ Zinc	0.15 $mg.l^{-1}$ Lead	0.30 $mg.l^{-1}$ Lead	0.50 $mg.l^{-1}$ Lead	0.15 mgl Zinc + 0.15 mgl^{-1} Lead	0.30 mgl^{-1} Zinc + 0.30 mgl^{-1} Lead	0.50 mgl^{-1} Zinc + 0.50 mgl^{-1} Lead	Gleneedle Stream Water Only	Glendhoo Stream Water Only	Totals
OBSERVED FREQUENCY f_o	40	31	20	42	34	20	38	20	0	42	36	323
EXPECTED FREQUENCY f_e	29.36	29.36	29.36	29.36	29.36	29.36	29.36	29.36	29.36	29.36	29.36	323

Data taken from fig. 6.2

Null Hypothesis, H_o = there is no difference in the number of days spent in pupation in each experimental solution.

$$\chi^2 = \sum \frac{(f_o - f_e)^2}{f_e} = 58 \text{ with 10 degrees of freedom}$$

The calculated value of is greater than the tabulated value of 23.2 (Fisher and Yates, 1963) therefore H_o is rejected at the 99% level and the difference between the number of days spent in pupation in each experimental solution is significant ($P<0.01$).

ZINC Concentration $mg.l^{-1}$			LEAD Concentration $mg.l^{-1}$			Percentage of Nymphs alive after 2 days	5 days	10 days	30 days	Maximum Period in days before a successful moult	Percentage of Nymphs successfully completing 1st moult	2nd moult
0.15	0.13	0.14	0	0	0	85(17)	80(16)	70(14)	45(9)	13	70(14)	45(9)
0	0	0	0.15	0.14	0.15	100(20)	85(17)	80(16)	45(9)	13	80(16)	45(9)
0.15	0.14	0.15	0.15	0.15	0.15	75(15)	70(14)	55(11)	20(4)	12	55(11)	10(2)
0.30	0.28	0.29	0	0	0	80(16)	70(14)	55(11)	20(4)	12	30(6)	10(2)
0	0	0	0.30	0.29	0.30	85(17)	80(16)	55(11)	20(4)	11	30(6)	5(1)
0.30	0.30	0.30	0.30	0.28	0.29	55(11)	45(9)	30(6)	10(2)	9	20(4)	0
0.50	0.47	0.49	0	0	0	65(13)	55(11)	40(8)	5(1)	10	15(3)	0
0	0	0	0.50	0.48	0.49	70(14)	60(12)	30(6)	15(3)	11	15(3)	0
0.50	0.48	0.49	0.50	0.47	0.49	45(9)	30(6)	15(3)	0	0	0	0
GLENDHOO STREAM WATER ONLY						90(18)	80(16)	50(10)	25(5)	12	60(12)	25(5)
CONTROL: GLENEEDLE STREAM WATER ONLY						100(20)	90(18)	85(17)	70(14)	14	80(16)	70(14)

TABLE A9. Observations of nymphs of <u>Protonemura meyeri</u>, 8-10 mm long, placed in water from the Gleneedle stream to which had been added different concentrations of salts of lead and zinc in the laboratory. Figures in brackets refer to actual numbers. 1st moult refers to 1st observed moult in the laboratory.

Calculation of the significance and association between the number of survivors of nymphs of <u>Protonemura meyeri</u> exposed to different concentrations of lead and zinc.

OBSERVED FREQUENCIES f_o						EXPECTED FREQUENCIES f_e				
Metal Concentration	Number of nymphs alive after 2 days	5 days	10 days	30 days	Row Totals	Number of nymphs alive after 2 days	5 days	10 days	30 days	Row Totals
0.15 $mg.l^{-1}$ ZINC	85	80	70	45	280	98	86	65	31	280
0.15 $mg.l^{-1}$ LEAD	100	85	80	45	310	109	95	72	34	310
0.15 $mg.l^{-1}$ ZINC + 0.15 $mg.l^{-1}$ LEAD	75	70	55	20	220	77	68	51	24	220
0.30 $mg.l^{-1}$ ZINC	80	70	55	20	225	79	69	52	25	225
0.30 $mg.l^{-1}$ LEAD	85	80	55	20	240	84	74	60	26	244
0.30 $mg.l^{-1}$ ZINC + 0.30 $mg.l^{-1}$ LEAD	55	45	30	10	140	49	43	33	15	140
0.50 $mg.l^{-1}$ ZINC	65	55	40	5	165	58	51	38	18	165
0.50 $mg.l^{-1}$ LEAD	70	60	30	5	165	58	51	38	18	165
0.50 $mg.l^{-1}$ ZINC + 0.50 $mg.l^{-1}$ LEAD	45	30	15	0	90	32	28	21	10	91
GLENDHOO STREAM WATER ONLY	90	80	50	25	245	86	75	57	27	245
CONTROL: GLENEEDLE STREAM WATER ONLY	100	90	85	70	345	121	106	80	38	345
COLUMN TOTALS	850	745	565	265	2425	851	746	567	266	2430

Slight discrepancies between the totals for f_o and f_e are due to 'rounding off' to the nearest integer.

Expected frequency = $\frac{\text{row total x column total}}{\text{grand total}}$

$$\chi^2 = \sum \frac{(f_o\ f_e)^2}{f_e}$$

= 100

with (11-1)(4-1) = 30 degrees of freedom.

Null hypothesis H_o = there is no difference in the number of survivors for each experimental condition.
The calculated value of χ^2 is considerably higher than the tabulated value of 50.892 (Fisher and Yates, 1963) therefore H_o is rejected at the 0.1% level and the difference in the number of survivors in each experimental condition is significant (P<0.01).

ZINC Concentration $mg.l^{-1}$			LEAD Concentration $mg.l^{-1}$			Percentage of Nymphs alive after				Maximum Period in days to 1st Successful Moult	Percentage of Nymphs successfully completing	
Init.	Final	Mean	Init.	Final	Mean	2 days	5 days	10 days	20 days		1st Moult	2nd Moult
0.15	0.14	0.15	0	0	0	94 (47)	66 (33)	58 (29)	0	18	4(2)	0
0	0	0	0.15	0.13	0.14	92 (46)	62 (31)	58 (29)	0	17	6 (3)	2 (1)
0.15	0.13	0.14	0.15	0.14	0.15	58 (29)	44(22)	8 (4)	0	-	0	0
0.30	0.28	0.29	0	0	0	90 (45)	48 (24)	8 (4)	0	-	0	0
0	0	0	0.30	0.29	0.30	90 (45)	52 (26)	12 (6)	0	-	0	0
0.30	0.29	0.30	0.30	0.30	0.30	32 (16)	18 (9)	4(2)	0	-	0	0
0.50	0.48	0.49	0	0	0	42 (21)	0	0	0	-	0	0
0	0	0	0.50	0.48	0.49	36 (18)	0	0	0	-	0	0
0.50	0.48	0.49	0.50	0.49	0.50	14 (7)	0	0	0	-	0	0
GLENDHOO STREAM WATER ONLY						96 (48)	78 (39)	68 (34)	6 (3)	21	32 (16)	0
CONTROL: GLENEEDLE STREAM WATER ONLY						98 (49)	80 (40)	72 (36)	58 (29)	13	80 (40)	42 (21)

TABLE A10 Observations of nymphs of Baetis rhodani 6–8 mm long placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory. Each test comprised five samples of ten nymphs. Figures in brackets refer to actual numbers. 1st moult refers to 1st laboratory observed moult.

ZINC Concentration $mg.l^{-1}$			LEAD Concentration $mg.l^{-1}$			Percentage of Larvae alive after			
Init.	Final	Mean	Init.	Final	Mean	5 days	10 days	20 days	40 days
0.15	0.14	0.15	0	0	0	100 (10)	100 (10)	100 (10)	100 (10)
0	0	0	0.15	0.13	0.14	100 (10)	100 (10)	100 (10)	100 (10)
0.15	0.13	0.14	0.15	0.14	0.15	100 (10)	100 (10)	90 (9)	80 (8)
0.30	0.29	0.30	0	0	0	100 (10)	100 (10)	100 (10)	100 (10)
0	0	0	0.30	0.29	0.30	100 (10)	100 (10)	90 (9)	90(9)
0.30	0.30	0.30	0.30	0.28	0.29	100 (10)	90 (9)	90 (9)	90 (9)
0.50	0.49	0.50	0	0	0	100 (10)	90 (9)	80 (8)	80 (8)
0	0	0	0.50	0.50	0.50	100 (10)	100 (10)	90 (9)	80 (8)
0.50	0.48	0.49	0.50	0.49	0.50	100 (10)	100 (10)	90 (9)	70 (7)
GLENDHOO STREAM WATER ONLY						100 (10)	100 (10)	90 (9)	90 (9)
CONTROL: GLENEEDLE STREAM WATER ONLY						100 (10)	100 (10)	90 (9)	90 (9)

TABLE A11. Observations of Polycentropus flavomaculatus larvae approximately 15mm long placed in water from the Gleneedle stream to which had been added different concentrations of salts of lead and zinc in the laboratory. Figures in brackets refer to actual numbers. Each test comprised five samples of two larvae and was discontinued after forty days.

ZINC Concentration $mg.l^{-1}$			LEAD Concentration $mg.l^{-1}$			Number of eggs	Number of days to first hatching	CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER							
Initial	Final	Mean	Initial	Final	Mean			20 days	25 days	30 days	35 days	50 days	55 days	60 days	75 days
0.15	0.14	0.15	-	-	-	94	17	8	15	22	22	22	22	22	22
0.15	0.14	0,15	-	-	-	136	19	8	16	19	19	19	19	19	19
0.15	0.15	0.15	-	-	-	111	19	9	13	19	19	19	19	19	19
-	-	-	0.15	0.15	0.15	80	18	9	19	22	23	23	23	23	23
-	-	-	0.15	0.14	0.15	96	17	9	20	21	22	22	22	22	22
-	-	-	0.15	0.14	0.15	120	16	9	20	24	24	24	24	24	24
0.15	0.15	0.15	0.15	0.15	0.15	101	-	0	0	0	0	0	0	0	0
0.15	0.13	0.14	0.15	0.14	0.15	112	-	0	0	0	0	0	0	0	0
0.15	0.14	0.15	0.14	0.13	0.14	107	-	0	0	0	0	0	0	0	0
GLENDHOO STREAM WATER ONLY						88	-	0	0	0	0	0	0	0	0
						119	-	0	0	0	0	0	0	0	0
						121	-	0	0	0	0	0	0	0	0
CONTROL GLENEEDLE STREAM WATER ONLY						94	19	2	4	7	8	12	70	90	90
						105	19	2	3	6	10	14	73	95	96
						136	20	3	4	8	9	14	75	96	96

TABLE A12. Observations of the hatching success of eggs of Potamophylax latipennis fertilized artificially on 6th September, 1979 and placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory.

ZINC Concentration $mg.l^{-1}$			LEAD Concentration $mg.l^{-1}$			Number of eggs	Number of days to first hatching	CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
Initial	Final	Mean	Initial	Final	Mean			55 days	60 days	65 days	70 days
0.15	0.14	0.15	-	-	-	50	20	3	5	7	11
0.15	0.13	0.14	-	-	-	54	22	2	5	8	9
0.15	0.15	0.15	-	-	-	58	22	2	4	8	9
-	-	-	0.15	0.15	0.15	61	20	2	6	8	10
-	-	-	0.15	0.14	0.15	70	25	3	5	8	10
-	-	-	0.15	0.14	0.15	94	28	2	4	7	9
0.15	0.13	0.14	0.15	0.14	0.15	29	-	0	0	0	0
0.15	0.15	0.15	0.15	0.15	0.15	41	-	0	0	0	0
0.15	0.14	0.15	0.15	0.13	0.14	49	-	0	0	0	0
GLENDHOO STREAM WATER ONLY						36	-	0	0	0	0
						51	-	0	0	0	0
						72	-	0	0	0	0
CONTROL						32	53	16	42	91	91
GLENEEDLE STREAM WATER ONLY						41	50	14	40	87	88
						56	50	14	40	89	89

TABLE A13. Observations of the hatching success of eggs of Polycentropus flavomaculatus fertilized artificially on 5th August, 1979 and placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory.

ZINC Concentration mg.l^{-1}			LEAD Concentration mg.l^{-1}			Number of eggs	Number of days to first hatching	CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER			
Initial	Final	Mean	Initial	Final	Mean			40 days	45 days	50 days	60 days
0.15	0.14	0.15	-	-	-	110	23	2	3	3	10
0.15	0.15	0.15	-	-	-	149	25	3	4	5	9
0.15	0.15	0.15	-	-	-	184	24	2	4	4	8
-	-	-	0.15	0.13	0.14	97	20	3	5	7	9
-	-	-	0.15	0.15	0.15	124	22	3	5	6	9
-	-	-	0.15	0.14	0.15	164	22	4	6	6	8
0.15	0.13	0.14	0.15	0.14	0.15	105	-	0	0	0	0
0.15	0.14	0.15	0.15	0.15	0.15	114	-	0	0	0	0
0.15	0.15	0.15	0.15	0.14	0.15	134	-	0	0	0	0
						76	-	0	0	0	0
GLENDHOO STREAM WATER ONLY						111	-	0	0	0	0
						119	-	0	0	0	0
CONTROL						121	37	4	46	83	84
GLENEEDLE STREAM WATER ONLY						125	38	5	48	84	84
						130	38	7	50	84	85

TABLE A14. Observations of the hatching success of eggs of Hydropsyche instabilis fertilized artificially on the 15th June 1979 and placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory.

ZINC Concentration mg.l^{-1} Initial	Final	Mean	LEAD Concentration mg.l^{-1} Initial	Final	Mean	Number of eggs	Number of days to first hatching	CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER 35 days	40 days	45 days	50 days
0.15	0.13	0.14	-	-	-	23	19	2	4	9	10
0.15	0.14	0.15	-	-	-	29	19	2	5	7	10
0.15	0.15	0.15	-	-	-	42	20	2	4	8	11
-	-	-	0.15	0.14	0.15	56	20	2	5	8	10
-	-	-	0.15	0.14	0.15	89	20	2	4	9	9
-	-	-	0.15	0.15	0.15	121	22	2	4	8	10
0.15	0.14	0.15	0.15	0.14	0.15	18	-	0	0	0	0
0.15	0.13	0.14	0.15	0.14	0.15	31	-	0	0	0	0
0.15	0.15	0.15	0.15	0.14	0.15	49	-	0	0	0	0
GLENDHOO STREAM WATER ONLY						60	-	0	0	0	0
						83	-	0	0	0	0
						100	-	0	0	0	0
CONTROL						40	29	8	52	68	69
GLENEEDLE STREAM WATER ONLY						71	30	9	53	70	70
						90	29	8	53	69	69

TABLE A15. Observations of the hatching success of eggs of *Rhyacophila dorsalis* fertilized artificially on the 20th July, 1979 and placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory.

ZINC Concentration $mg.l^{-1}$ Initial	Final	Mean	LEAD Concentration $mg.l^{-1}$ Initial	Final	Mean	Number of eggs	Number of days to first hatching	CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER 50 days	55 days	60 days	65 days
0.15	0.14	0.15	-	-	-	121	30	4	4	4	4
0.15	0.14	0.15	-	-	-	139	28	4	4	4	4
0.15	0.15	0.15	-	-	-	151	29	2	2	3	4
-	-	-	0.15	0.14	0.15	103	27	2	2	2	3
-	-	-	0.15	0.14	0.15	142	26	2	2	2	3
-	-	-	0.15	0.14	0.15	194	26	1	1	2	2
0.15	0.14	0.15	0.15	0.15	0.15	98	-	0	0	0	0
0.15	0.15	0.15	0.15	0.14	0.15	119	-	0	0	0	0
0.15	0.15	0.15	0.15	0.15	0.15	146	-	0	0	0	0
						121	-	0	0	0	0
GLENDHOO STREAM WATER ONLY						136	-	0	0	0	0
						157	-	0	0	0	0
CONTROL						99	45	13	55	91	91
GLENEEDLE STREAM WATER ONLY						120	47	12	52	90	91
						142	47	12	51	91	91

TABLE A16. Observations of the hatching success of eggs of Baetis rhodani fertilized artificially on the 2nd August, 1979 and placed in water from the Gleneedle stream to which had been added different concentrations of the salts of lead and zinc in the laboratory.

ZINC Concentration $mg.l^{-1}$ Initial	Final	Mean	LEAD Concentration $mg.l^{-1}$ Initial	Final	Mean	Number of eggs	Number of days to first hatching	CUMULATIVE PERCENTAGE OF EGGS HATCHED AFTER 40 days	45 days	50 days	55 days	60 days
0.15	0.13	0.14	-	-	-	72	39	4	4	5	5	5
0.15	0.14	0.15	-	-	-	90	34	3	3	4	4	4
0.15	0.14	0.15	-	-	-	136	33	3	3	4	4	4
-	-	-	0.15	0.14	0.15	87	34	2	2	2	3	3
-	-	-	0.15	0.13	0.14	94	32	2	3	3	3	3
-	-	-	0.15	0.13	0.14	121	38	2	2	2	2	2
0.15	0.13	0.14	0.15	0.14	0.15	62	-	0	0	0	0	0
0.15	0.14	0.15	0.15	0.13	0.14	89	-	0	0	0	0	0
0.15	0.15	0.15	0.14	0.14	0.15	145	-	0	0	0	0	0
GLENDHOO STREAM WATER ONLY						70	-	0	0	0	0	0
						85	-	0	0	0	0	0
						105	-	0	0	0	0	0
CONTROL						76	48	0	0	8	67	81
GLENEEDLE STREAM WATER ONLY						84	51	0	0	0	64	80
						95	50	0	0	4	63	80

TABLE A17. Observations of the hatching success of eggs of Protonemura meyeri fertilized artificially on the 12th May, 1979 and placed in water from the Gleneedle stream to which had been added different concentrations of salts of lead and zinc in the laboratory.

RIVER	METAL CONCENTRATION $mg.l^{-1}$ LEAD	ZINC	RATIO LEAD/ZINC	MEAN RATIO LEAD/ZINC	VARIANCE
YSTWYTH	0.005	0.015	0.33	0.13	0.10
	0.067	0.565	0.12		
	0.050	0.447	0.11		
	0.032	0.386	0.08		
	0.098	2.002	0.05		
	0.028	0.411	0.07		
RHEIDOL	0.010	0.336	0.03	0.09	0.10
	0.004	0.012	0.33		
	0.005	0.147	0.03		
	0.010	0.104	0.10		
	0.012	0.296	0.04		
	0.009	0.327	0.03		
	0.010	0.159	0.06		

TABLE A18. Ratio of the concentration of lead to the concentration of zinc at various sites on the rivers Ystwyth and Rheidol. (Values are calculated using metal concentrations recorded by Brooker and Morris, 1980).